A Summary of Current Program 7/1/65

and Preliminary Report of Progress

for 7/1/64 to 6/30/65

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WESTERN UTILIZATION RESEARCH AND

DEVELOPMENT DIVISION

of the

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

and related work of the

STATE AGRICULTURAL EXPERIMENT STATIONS

This progress report is primarily a tool for use of scientists and administrators in program coordination, development and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of progress on USDA and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of USDA and cooperative research issued between July 1, 1964, and June 30, 1965. Current agricultural research findings are also published in the monthly USDA publication, Agricultural Research. This progress report was compiled in the Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Albany, California.

UNITED STATES DEPARTMENT OF AGRICULTURE

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INTRODUCTION

Utilization research in agriculture deals with the discovery and development of new and improved products and the invention or perfection of processing technologies. The scientists, engineers, and technologists who carry on this research devote much of their effort to basic studies of physical and chemical properties of agricultural commodities and products derived from them, in order to provide a firm base of exact knowledge for applied developments.

The present report summarizes the current research program of the Western Utilization Research and Development Division (one of four Utilization Divisions in the Agricultural Research Service) and of the State Agricultural Experiment Stations in the areas reported, makes a report of progress toward the objectives of the Federal program during Fiscal Year 1965, and describes a few of the more significant recent accomplishments of this work.

Research Area Covered by this Report

The farm commodities dealt with in this report are the cereal grains, wheat, rice, and barley; alfalfa and other forage crops; wool and mohair; citrus, apples, other fruits, and tree nuts; potatoes and other vegetables and dry beans and peas; castor and safflower; sugar beets; new and replacement crops; and poultry and eggs. Some phases of research on certain of these commodities are pursued in other Utilization Research Divisions than the Western Division: Research on industrial uses of wheat and on milling technology is carried on in the Northern Division; certain areas of research on deciduous fruits and on potatoes and other vegetables are handled by the Eastern Division; particular lines of research on rice, vegetables, and fruits are carried on in the Southern Division. Research on new and replacement crops is carried on in all four Utilization Divisions.

Pharmacological research for all four of the Utilization Research Divisions is conducted at the Albany laboratory of the Western Division, and is described in this report.

Aims of Research on These Commodities

The group of commodities discussed here provides the nation with more than half of its food, either directly (cereal grains, fruits and vegetables, poultry meat and eggs, and beet sugar) or indirectly through feeding of meat animals (forage crops, wheat, barley). The other commodities in the group supply us with our most important animal fibers (wool and mohair) and offer opportunities of development into numberless industrial products (castor, safflower, and other oilseeds).

The general aim of utilization research on both of the two broad categories of farm commodities is essentially the same--to broaden and extend utilization of the commodities and thereby help to stabilize or increase the demand

for them. The scientific procedures of research are broadly the same in both areas, whereas the technologies are in many respects different, especially as between the food materials and all the others. The fundamental justification for carrying on a publicly supported program of utilization research on food products follows a somewhat different line of reasoning than the justification for research to extend the utilization of non-food commodities.

Research toward utilization of non-food products may be based on the avowed public policy of assisting farmers threatened with loss of markets as a result of the swift rise of non-agricultural synthetics, as in the case of wool growers; or on the possibility of developing demand for a presently minor crop to the point where it can be grown profitably on a very large scale in order to remove some of the pressure of surplus from other crops--for example, the development of castor and certain other industrial oilseeds which take land out of cotton and feed grain production.

Research on the processing of farm products for food, on the other hand, is justified primarily by its direct benefit to the entire population through improved nutrition and well-being, reduction of economic losses resulting from spoilage and waste, and increased opportunity to find profitable markets abroad. Indirectly, too, advances in technology through food processing research bring about major and desirable shifts in the commodity supply and demand picture for the country as a whole, as for example in the economical conversion of abundant feed grains into broiler-type chickens, marketable in refrigerated or frozen form throughout the nation and stabilization of dehydrated alfalfa that makes it suitable for export.

Organization of the Division

Research and development along these diverse lines are carried on for the Western Division by a staff headquartered in the Western Regional Research Laboratory, Albany, California. A smaller Department-owned laboratory is operated in Pasadena, California; laboratory space and facilities in Puyallup, Washington are utilized through a cooperative arrangement with Washington State University, Institute of Agricultural Sciences; and laboratory space and facilities in Honolulu, through a cooperative arrangement with the University of Hawaii.

The Albany research staff is organized into six commodity-oriented Laboratories (Cereals, Field Crops, Fruit, Poultry Products, Vegetables, and Wool and Mohair); two functional Laboratories (Pharmacology, and Engineering and Development); and a Pioneering Laboratory concerned with basic studies of plant enzymes. The staff at Pasadena is organized as the Subtropical Fruit Laboratory. The Western Regional Research Laboratory at Albany also houses the Division Director's staff, the staff required for Administrative support of the Division, and that responsible for Plant Management--that is, operation of the buildings, facilities, and grounds.

Division scientists and engineers not only conduct or supervise research in their own experimental facilities, but also greatly extend the scope and

influence of their work by planning and supervising developmental activities carried on by cooperating private firms, processor organizations, or industry groups, and by arranging for research by well-qualified scientists elsewhere under research contracts. In addition, certain grants of research funds are placed with investigators in foreign countries; the cost of these foreign research efforts on behalf of American agricultural interests is borne by Public Law 480 funds.

Examples of Recent Accomplishments of the Western Utilization Research and Development Division

WURLD Wheat Attracts Interest Both in the United States and in Asia by its Whiteness, Digestibility, Nutrition, Convenience, Economy and Stability. Cooked whole kernel-form wheat, free of colored bran, has been called WURLD Wheat by its USDA inventors. The name is reminiscent of the new food's acceptability throughout the world, as well as suggesting the process, in which the lye treated bran is whirled off in a turbulent water stream before acid treatment. A pilot plant, continuous and automatic, has been built to determine best conditions for future installations. The plant, rated at 250 lbs. per hour, has been used to make several tons of WURLD Wheat, which was tested in Hong Kong by Church World Services. Acceptability of the product was found to be outstanding. Industry raised several objections to the earlier process. These were high water consumption, unsalable bran by-products, and difficult effluent disposal. All these objections have now been overcome. Better lye treatment has reduced water use to 8 lbs. per lb. of wheat, produced a compressable bran which can be used in feeds, and an effluent capable of biological reduction to a harmless state. These improvements have also markedly reduced the cost of the process. The versatility of the process has been further demonstrated on rice, barley, oats, peas, milo, and sesame.

New High Protein Beverage Products from Wheat. Easily dispersible, high protein powders have been developed from wheat materials for overseas markets where protein-rich foods are scarce. They are readily usable as beverages resembling milk, and can also be easily used for making rich soup or pudding mixes. This versatility is possible because the base products are quite bland, and can thus accept any pattern of flavorings, seasonings, and mineral or vitamin enrichment which may be necessary to meet the needs and preferences in any given foreign country. In making these powders, combined enzymatic and acid hydrolysis treatments are used to bring about carefully graded solubilization of the naturally insoluble protein and starch in any type of wheat flour. Thus, protein contents can be adjusted to any desired level, and stabilized vegetable fats can be added to adjust caloric values and to enhance palatability. As part of the process, high biological value protein is extracted from the bran and is blended back into the flour. This improves the nutritive quality of the protein. It is also possible to add non-fat dry milk solids, as available, to the product to increase its lysine content. Laboratory processes for making the new products are now being scaled-up to commercial type processes.

High Protein Rice Flours. Department scientists have shown that substantial quantities of rice flours of up to 16 to 20% protein can be abraded from the surfaces of either ordinary or parboiled milled rice. Currently available types of milling machinery are used, and thus the cost of producing the flour is quite low. If only three to five percent of the starting rice is milled off, breakage is negligible and the residual kernels are whiter and cook better. The color improvement is especially prominent with parboiled rice. The rice thus has a slightly higher sales value, perhaps high enough to offset the cost of producing the flour. The bland flours are non-allergenic, low in fiber, and provide high quality protein for infants, for adults with high blood pressure, and for elderly people who have dietary problems with normal sources of food protein. A large potential export outlet also exists to supply protein for weanling children in developing countries. The cooked flours from parboiled rice can be used directly to make gruels or they can be converted to powdered, soluble beverages in this country and then sold overseas. Further studies are being undertaken in cooperation with UNICEF and with industry to incorporate these flours into new products such as baby foods, beverages and soups.

Cooperative Work with Industry Provides Knowledge of Dehydrated Alfalfa Needed for Computing Least-Cost Feed Formulations. Department studies are leading to markedly improved feed materials at lower costs. The use of computers to calculate least-cost feed formulations has expanded greatly in the past several years. The proportions of each feed ingredient to be used are based on the relative costs of all the important nutrients available from competitive sources. Such complex calculations were impossible in pre-computer days. Precise analytical data for all nutrients in processed feedstuffs is a prime requirement, but such data have not been generally available. Furthermore, adequate analytical methods for alfalfa products were lacking in some cases. To solve this problem, a cooperative basic research effort was undertaken by the Department's Western Utilization Division, two universities, a major feed company, a research institute, and the American Dehydrators Association. Dehydrated alfalfa from all major production areas of the country was subjected to exhaustive chemical analyses and metabolizable energy assays. The first of a series of bulletins, containing only a part of the results, reached every segment of the industry within the first month after issue. Thus, an important gap in basic knowledge is being filled and this knowledge is being used by industry to make effective use of alfalfa meals in minimum cost feeds. The research is now being extended to other forage crops.

Mycotoxins Found To Be the Causative Agents of Fescue Foot. A number of toxigenic molds have been isolated from tall fescue. Their various toxic effects have been demonstrated in the mouse, rabbit and sheep by using suitable extracts of either the toxic hays or pure cultures of molds isolated from the hay. In the rabbit, topical application of extracts on the unabraded skin produces hyperemia, edema, hemmorhage and death. In the mouse, intraperitoneal injections of clear filtrates of submerged cultures of the molds produce death as a result of massive pulmonary and visceral hemorrhage. Force feeding of total mold cultures to a mature ewe produced total ruminal paralysis.

This latter result is comparable to the clinical pathology of cattle suffering fescue foot, in which death results from the ensuing starvation and dehydration. Three of the active metabolites of one of the toxic molds (Fusaria) have been isolated, and these are capable of producing the same toxic effects in rabbits and mice as the original hay or mold extract. These substances are being characterized chemically and evaluated in cooperative tests on cattle.

New Soil Repellent Finishes for Wool. Fluoropolymers provide the most effective soil-repellent treatments for textiles known to date, but these finishes hitherto have been exceedingly expensive. Now an entirely new synthesis has been discovered which gives promise of providing superior soil-repellent finishes at less than half of the cost of previously available treatments. From hexafluoroacetone, several new families of polymers have been made. These include polyfluoroacrylates, polyfluoroethers and others. The new polymers, dissolved in proper solvents, can be easily applied to fabrics or finished garments to provide water- and soil-repellent finishes which are durable through repeated laundering, drycleaning and wear. Some of these new fluoropolymers give wool shrink resistance in addition to soil repellency and thus open the way to new multipurpose finishes for wool. They can also be used in combination with the WURLAN treatment, which is now being extensively used commercially to shrink-proof wool so that it can be machine-laundered.

Formaldehyde Stops Yellowing of Moist Wool. A new formaldehyde treatment protects raw wool from the yellowing and consequent economic losses which occur when fleece is shown and baled under adverse conditions. If the moisture content of the raw wool is over 30%, the temperature within the bales rises as much as 40° F. within a week. Yellowing starts at once and increases for many weeks. Both the yellowing and the temperature rise are associated with a combined chemical and microbial action. It has now been discovered that both can be prevented if the raw wool is sprayed with a dilute formaldehyde solution or is lightly dusted with paraformaldehyde prior to baling. This new treatment will save money by eliminating a bleaching step in processing, avoid the consequent fiber weakening, and maintain the higher price which white wool commands. Avoidance of bleaching is also desirable because, while it may temporarily whiten the wool, the yellow color often reappears during later use, especially if the wool is washed in hot water.

Bitter Limonin Discovered in Grapefruit Juice. Excessive bitterness is one of the main factors that limits the consumer acceptability of grapefruit juice. In the past, grapefruit bitterness was ascribed to a substance called naringin, whereas the bitterness of navel orange juice is due to another compound, limonin. Department scientists have now discovered that limonin also occurs in grapefruit juice in amounts sufficient (up to 10 parts per million) to contribute significantly to the bitterness of the juice. Limonin has been found both in commercial grapefruit juice products and in juice prepared in the laboratory from fresh fruit grown in Arizona, California, Florida, and Texas. Limonin is located primarily in the pulp of the fruit and enters the juice during extraction. The knowledge that limonin, as well as naringin,

contributes significantly to the bitterness of grapefruit provides us for the first time with a sound basis for developing a complete debittering process.

Process for Preservation of Soft and Easy-to-Handle Raisin Paste. Department scientists have made it possible to use surplus raisins for making raisin paste which promises to be a large new outlet. Raisin paste normally sets into a hard, crystalline mass after only a few weeks' storage. Now a new process has been developed which prevents this hardening by using a heat treatment that requires only a few seconds. Raisin paste prepared in this way remains soft and pliable for months. The treated paste can be economically produced and distributed to the institutional food and bakery trade, where it can be incorporated into such items as filled cookies, coffee cakes, pies, tarts, etc. Delicious products were obtained by a commercial bakery in an experimental lot of various items containing treated raisin paste. The new process is being evaluated by the California raisin industry. The research has had partial financial support from the Dried Fruit Association of California.

New Process for Manufacturing Quick-Cooking Dry Beans. Production of dry beans has increased recently, tending to increase an already abundant supply. A new process for making a convenient quick-cooking product promises relief from this surplus. Although dry beans are a rich source of low-cost protein, the modern housewife is often unwilling to spend the time and effort required to prepare beans in the conventional manner. (One of the major reasons is the long soaking time which is necessary.) The California Lima Bean Advisory Board was most anxious to see a quick-cooking bean developed, and has been providing partial financial support for the research on Lima beans. A practical process to produce quick-cooking, essentially non-flatulent, dry Lima beans has now been developed. The processed beans may be prepared for table use in about 30 minutes by simply cooking them in boiling water; no soaking is necessary. In contrast, untreated beans normally require about 15 hours soaking, followed by 1 to 2 hours cooking. The quick-cooking beans have a normal appearance and improved flavor. Several food manufacturers are interested in using the process on several varieties of dry beans.

New Method to Reduce Heat Resistance of Bacterial Spores. In canning non-acid foods, it is necessary to use severe heat treatments in order to destroy bacterial spores, which are remarkably heat resistant. If a way can be found to substantially reduce the required heating time, it would be possible to produce canned vegetables of markedly improved color, flavor, nutritive value, and acceptability. In the course of a basic research project aimed at understanding how spores resist destruction by heat, Department scientists discovered a previously unrecognized property of spores which appears to control their heat resistance. Based upon an understanding of this property, it is now possible to alter the heat sensitivity of spores by a simple chemical treatment. Efforts are now being made to use this information to devise a method to greatly reduce the heating required for sterilization. If these efforts succeed, it may be possible to develop improved products from many non-acid vegetables, such as cauliflower, sweet corn, wax beans, and squash.

New Onion Pungency Test Adopted by Industry. Onions are the sixth largest vegetable crop grown in the U.S. with a farm value of $85 million. Because onions are used in foods largely for their appealing pungency, the value of dehydrated onions is directly related to their strength of flavor. Thus, it is important that processors be able to measure pungency, but this has been difficult to do because trained taste panels must be available. Basic research by Department scientists has now led to a new objective method for measuring flavor strength of onions. They discovered the principal chemical reaction responsible for flavor development when onion cells are crushed, showed that the quantity of pyruvic acid produced by this reaction is directly related to pungency, and then developed a test to measure the pyruvic acid. This achievement has been adopted by the domestic dehydration industry for selecting raw materials for processing, and by plant breeders to obtain varieties of onions more suitable for processing. In addition, one large user of dehydrated onions specifies purchases according to this test. This Department research is thus resulting in improved products for U.S. consumers.

Broadened Usage of Castor Oil is Expected as a Result of Methods for the Production of Low-Cost, Non-Burning, Rigid Polyurethane Foams. The construction industry is a potential yearly outlet for as much as one billion pounds of plastics for insulation and vapor barriers. Polyurethane foams with their unique property of on-site fabrication should realize a large share of this market as they become accepted by the building industry. A reactive liquid mixture can be poured into voids (e.g., roof, floor, or wall members); it foams up, filling the void, and provides rigidity and strength, as well as a heat, sound, and vapor barrier. Such foams are now mainly based on petrochemicals, but Department research has demonstrated that modified castor oil can be used to make polyurethane foams. These foams can be made flame-resistant with no loss in other properties by incorporation of reactive flame-resistant chemicals. The least flammable castor oil based foams are those prepared in one-step ("one-shot") systems from brominated castor oil. These completely non-burning foams are significantly less expensive than similar fire-resistant foams based on petrochemicals. Based on the results of this Department research, several large fatty acid processors have initiated development programs in this field. Large increases in castor oil could result, requiring up to 200,000 new acres of castor.

Development of a Rapid Method of Analyzing Egg Products for Salmonella Using the Fluorescent Antibody Technique. The presence of Salmonella in food products and in raw materials can now be detected quickly and easily as a result of Department research. Other currently used assay methods for detecting Salmonella in eggs and egg products are both tedious and time consuming. Complete assays require from three to five days. A fluorescent antibody technique has been applied to the development of an assay method for Salmonella in egg products that can be completed in 10 to 24 hours. In this method specific Salmonella antibodies, obtained from rabbits and guinea pigs, and labeled with fluorescent dyes, become concentrated around any Salmonella cells present and appear as fluorescent spots on microscope slides. Other microorganisms do not react with the antibody, and no fluorescent spots are evident. The new method compares favorably with existing

methods both in reliability and sensitivity and represents another step in our drive to eliminate Salmonella from food products.

Superior New Frozen Foods in Ready-Made Sauces. Several years ago Department scientists discovered methods for stabilizing to freezing, starch- and egg-thickened sauces, gravies, salad dressings and desserts. These methods overcame the undesirable texture changes occurring in frozen foods, which are characterized by objectionable liquid separation and a curdled appearance after thawing. In the case of emulsion-containing foods such as salad dressings, freezing caused the emulsion to break down. The demonstration that these problems could be overcome by replacing the usual thickening agents either wholly or in part by waxy rice flour, along with use of vegetable oils which do not crystallize, has formed a basis for the development of entirely new frozen food products having superior quality. A large number of "boil-in-the-bag" items are now available in U.S. markets. Sales of vegetables containing stable sauces, and meats containing stable gravies, are already over the $200 million mark, and it is estimated that there will be a five-fold increase by 1968. A recent "Consumer Reports" survey describing studies on boil-in-the-bag vegetables with butter sauce states "the taste-testers judged the quality of the boil-in-the-bag products to be about the same as that of the homemade butter-sauced vegetables."

Chlorogenic Acid, a Component of all Higher Plants, Is Not an Allergen. Department scientists have refuted the claim that chlorogenic acid is a potent food and respiratory allergen. Chlorogenic acid occurs in many foods including potatoes, carrots, cabbage, coffee, apples, and peaches. Because it is so widespread, it could be a most troublesome compound if it were allergenic. Department scientists, with clinical collaboration of a French allergist, have now demonstrated conclusively that chlorogenic acid has no role in human allergy. This finding was made possible by use of a new ARS-developed test for allergy in which monkeys are used to detect reaction of suspected allergens with sera of allergic patients. Allergy to coffee, for example, was shown to be caused not by reaction to its chlorogenic acid content, but to protein present in the green coffee bean. Similarly in cases of allergy to other plant materials, it was shown that specific proteins, rather than chlorogenic acid, were the responsible allergens. These studies show that food plants and other agricultural products present no allergenic hazard attributable to the presence of chlorogenic acid.

AREA NO. 1. WHEAT AND BARLEY-- FOOD AND FEED PRODUCTS AND PROCESSING

Problem. The dominant factor in the wheat economy of the United States continues to be that production capacity has outpaced even the substantially expanded foreign markets of recent years. Supply and demand have been brought closer to balance only through production curtailment and substantially subsidized foreign marketing. The resulting tendency to depress the agricultural economy has an important influence on the entire national economy. Despite the shrinkage in numbers of the farm population in the past 20 years, agriculture remains the principal ultimate customer of our nation's industrial products and services. Agriculture also provides the largest share of our commercial exports and contributions to a favorable trade balance. Wheat is a major commodity in both our domestic and foreign trade considerations, and is a primary segment of our agricultural economy. Sustained further gains in wheat markets are necessary to ease restrictions in production and to strengthen trade balances. Especially needed are increased domestic usage and commercial exports. Domestic usage of wheat has been relatively constant for many years, but encouraging signs of a stabilized per capita food consumption and a trend for greater feed usage in the past two years suggest that total domestic usage can soon begin to exceed the prevailing 600 million bushel total. For the past several years exports have surpassed domestic usage, but a large proportion of the exports have been concessional sales and donations with long-range market-building objectives. Increased world supplies of wheat and restrictive political decisions in the European Economic Community have contributed to seriously reduced commercial exports during the past marketing year. Everything possible must be done to increase total wheat markets, but especially those in which payments are made in dollars. The export donations and concessional sales in excess of 500 million bushels provide food where it is most needed in the world and serve immediate Defense and State Department missions, as well as the long-range market development for U.S. agriculture. The less than 200 million bushel commercial export of wheat, however, is the most important segment of the market to be increased. New processes to elicit maximum quality performance of wheats and flours in national products of all markets will help significantly in this regard. A more efficient conversion of wheat, mill feeds, and barley to meat will offer an improved opportunity to use more of the grains, although at a lower return to growers than from food products. New wheat food products specifically adapted to conditions of use in every region of the world will help materially in popularizing this valuable food grain in areas where it is now virtually unknown. Development of simplified methods to process the products at point of use will speed adoption of the many ways wheat can be used as food. An essential foundation for a successful product and process development program is a strong program of basic research on the composition of all classes of wheat and the fundamental properties of their constituents.

USDA AND COOPERATIVE PROGRAM

Research on utilization of wheat and barley for food and feed seeks to solve the most urgent problems hindering the development of markets for the full productive capacity of U.S. agriculture. The emphasis is on (1) expansion of overseas dollar markets for U.S. wheats; (2) development of new wheat food products for long-term market development in food-short nations abroad; (3) raising the domestic consumption of wheat foods by increased variety, quality, and convenience; and (4) finding means to upgrade wheat millfeeds and barley so they will be more valuable as livestock and poultry feeds. Basic research on the fundamental chemical and physical properties of wheat and barley constituents and on the functional properties of wheat flour constituents supports the product development and problem-solving segments of the program.

The long-standing and widespread popularity of wheat as a food substance depends chiefly upon the unique properties of its proteins, but adequate understanding of how these proteins interact with one another and with the other important flour constituents to bring about desired properties in wheat foods has not yet been achieved. Despite centuries of skillful empirical use of wheat in milling and baking, quality problems remain to impair the efficiency of using this valuable food material, particularly as changes in technology come along in the processing industries. Product development likewise is slowed by lack of adequate fundamental information on properties of components. Basic chemical studies are therefore conducted on wheat proteins, lipids, carbohydrates, and enzymes and on specific interactions among these components. Chemical changes that occur during the artificial maturing of flours or that occur during the mixing of doughs are studied to learn how wheats differ from one another, how each should best be processed, and how inherent quality variations can best be accommodated in desirably standardized and mechanized processing. Such considerations are also very important for our dollar wheat markets overseas because in the more important, such as Japan and Western Europe, highly mechanized production of bakery products is increasing as it has in the U.S. New methods for the artificial maturing of hard red winter wheat flours are especially needed for the European markets because methods commonly used elsewhere are not allowed and because as strong a baking performance as possible is needed to meet the competition from the high-quality wheats marketed from Canada. Our search for new maturing methods requires an understanding of the complex mechanisms of the process which involve enzymes, sulfhydryl groups on proteins, unsaturated fatty acids, and atmospheric oxygen, among other factors. Fundamental work of this type also aids in the development of new wheat food products being designed for varying needs in specific foreign countries. Development is well along, for example, in the enzymatic conversion to soluble form of the protein of wheat and wheat materials for high-protein beverage products suitable for infant feeding abroad. Other work involves development of high-protein formulations resembling meat products in flavor and texture, again for markets overseas where protein supplies are scarce.

Research is also continuing on development of inexpensive, easily processed types of products related to bulgur, such as WURLD wheat and malted products to help meet both calorie and protein needs in developing countries and to help build future dollar markets for U.S. wheats.

Several lines of work are primarily concerned with improving the quality and variety of wheat foods for the high-return domestic market. Such work includes a continuing study of the mysteries of the flavor and aroma of freshly baked bread so that product quality can be kept up to par or improved as economic factors force changes in baking and distribution methods. Related work seeks to overcome the inability of many flours to carry the amount of nonfat dry milk customarily and desirably used in breads when they are produced by the increasingly important continuous-mix methods. Work is being started on the fermentation aspects of bread production, not only to improve control of flavor production, but also to develop frozen doughs of better quality and stability. Several types of snack and convenience foods based on WURLD wheat and malted products are being developed to broaden the base of wheat food offerings to consumers.

Increased attention is being devoted to better utilization of the large tonnages of feed products from flour milling now marketed at income depressing prices. Methods are under development to recover the high biological value protein from millfeeds for use in food products and to make energy components more easily utilizable by poultry and livestock. Factors affecting the biological availabilities of protein and energy constituents of wheat feeds are being measured to assist their attainment of a better competitive position in modern feed processing based on linear programming and computerized formulations.

Research is conducted by the Western Utilization Research and Development Division at Albany, California; under contracts and grants at Pullman, Washington; Chicago, Illinois; Manhattan, Kansas; Madison, Wisconsin; St. Paul, Minnesota; Menlo Park, California; and Corvallis, Oregon; and under P.L. 480 grants in England, France, Poland, Italy, Australia, Switzerland, and Israel.

The Federal program of research in this area totals 36.7 professional man-years, including one scientist whose salary is provided by the Farmers Co-Operative Commission Company under a Memorandum of Understanding and 10 contracts and grants providing research at a rate of approximately 8.6 professional man-years per year. Of this number, 20.0 are assigned to investigations on chemical composition and physical properties; 14.5 on new and improved food products and processing technology; and 2.2 on new and improved feed products and processing technology. In addition, the Division sponsors 15 research grants under Public Law 480 including 13 on basic studies and 2 on applications of research.

PROGRAM OF STATE EXPERIMENT STATIONS

State stations conduct a comprehensive program of basic and applied research directed to increasing or improving the utilization of wheat and barley. These studies involve determining the influence of environmental, agronomic, harvesting and storage factors on the ultimate milling and baking quality of new selections and established varieties. Physical dough properties and baking characteristics are evaluated to provide guidance to cereal breeding programs and full knowledge of quality to the milling and baking industry. Testing often begins with laboratory micro-quality tests and extends through full-scale milling treatment.

The newer types of malting barley are studied to determine adaptability for malting. Basic composition, including enzyme content, is followed and these properties are related to potential for commercial malting.

The unique value of wheat flour for baking is due to special characteristics of the proteins. By labeling these proteins with radioactivity, the changes which take place during the various steps of baking are evaluated. Powerful new electrophoretic methods are being used to show that flour contains many proteins rather than the three or four previously thought present. Analytical techniques are being developed to determine quantitatively the protein content of wheat flour samples. These can also be used to develop wheat varieties containing more of the desirable proteins. Development of higher protein quality would improve the value of wheat in the diets of underdeveloped countries. For example, detailed research on the amino acid content of wheat, especially lysine, provides information of wide interest from a nutrition standpoint.

A number of basic studies are being carried out to further elucidate fundamental principles involved in conversion of cereals into food products. These include determination of physical grain properties, physical properties of small particles important in flour milling technology, and the nature of the enzymes, lipids and water soluble gluten fractions. Hydrogen bonding in proteins is being studied to clarify its role in determining the baking quality of wheat proteins.

The aroma and flavor of fresh bread and bakery products have universal appeal. Researches directed to determining the constituents responsible for the odor and flavor of bread and bakery products continue. Modern techniques such as gas chromatography are being used in these studies.

As a part of a broad study on conditioning of wheat, the changes in some properties of the aleurone cell layer caused by steam-conditioning are being investigated. Present data indicate denaturation of the proteins of the aleurone cell layer may be a major change brought about by steam-conditioning.

Although application of bleaching and improving agents to flours has been practiced for many years by the industry, the precise role played by chlorine

in the bleaching process has not been elucidated. Researchers are applying the tool of x-ray fluorescence spectroscopy for the analysis of the chlorine content of unbleached and bleached flours and their major biochemical fractions. The mechanism of improver action in cake flours is also being studied by determining the relation between flour specific surface and chlorine distribution. Other studies involve problems of air classification, the preparation of fine and intermediate fractions of wheat flour and the structure of the starchy endosperm.

Attempts to develop new and improved cereal products are directed to determining the fundamental properties of doughs and the effects of freezing upon them. Stability tests and related investigations on a wheat wafer for shelter rations are being conducted in cooperation with USDA (WU,ARS). Survival rations, including cereal products, are being evaluated.

Economic feasibility studies give attention to use patterns of barley and wheat of differing quality and the supply and flow patterns of wheat.

The total research effort on wheat and barley utilization research is approximately 12.2 professional man years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Proteins of Wheat. Protein quantity and quality, more than any other compositional factors, determine the bread-making and many other baking qualities of wheat flour. Mixing and kneading transform flour paste into strong, smooth, resilient dough. The protein of the flour determines whether or not the dough will be elastic enough to retain its bubbles of CO_2 as the yeast ferments and at the same time be fluid enough for the bubbles to expand and form a bold, attractive loaf. The spread of cookies, the formation of cake crumb, and the texture of crackers also depend considerably upon the amount and character of wheat protein although each demands differences in these factors from those ideal for bread making.

Bonds are made and broken within and between protein molecules as bread dough is worked. Non-protein wheat constituents and other dough ingredients affect the making and breaking of protein bonds. The rate and extent of all the reactions determine the quality, shape and size of the baked loaf. The association, interaction, or aggregation of flour proteins and the significance of such behavior to the functional properties of doughs and batters comprise an important area of utilization research. Increased knowledge of these factors is necessary for significant new advancements in product improvement, development of new products, and greater usefulness of U.S. wheats in foreign dollar markets.

A new concept emanating from our research indicates that protein particles must disaggregate during mixing before an appreciable protein network or

film can form. Such a network or film is widely regarded as the essential role of wheat protein in the development of strong bread doughs. Differences in the size and stability of protein aggregates of flour prior to mixing may explain the wide differences among flours as to their dough-mixing requirements. In order to test our ideas in this area, we have separated small amounts of wheat flour components and groups of components that can be used for baking or other tests in reconstituted doughs or simpler systems.

When a strong, high-quality flour is treated with dilute acetic acid, the residue is a water-insoluble protein-carbohydrate mixture in the form of a highly hydrated gelatinous mass. If the same extraction procedure is applied to doughs, the amount of this residue is less, and it decreases rapidly with additional mixing. The insoluble material, which consists of a protein that is strongly associated with hexosan and pentosan materials, appears to be a major factor governing the mixing requirements of flours, and possibly their baking performance. The increasing solubility that accompanies mixing is probably related to separation of the associated carbohydrate and protein.

Gluten is the major protein substance that provides flour with the functional characteristics associated with good baking quality. We are investigating the effects of both crude and purified gluten preparations on the behavior of doughs. The proportion of aggregated protein in the gluten preparations was lowered by dispersing them in dilute acetic acid in a blender or by centrifuging them to remove aggregates. Doughs containing crude glutens required the longest mixing times to reach maximum resistance. Doughs containing gluten subjected to the dispersing and centrifuging treatments required much less time. These results are consistent with the assumption that the protein aggregates present in flour must be disaggregated in the dough-mixing process before film formation can occur.

Gluten can be separated into glutenin and gliadin. Gliadin components are relatively small molecules (molecular weight about 25,000) and thus cannot be expected to contribute significantly to viscoelastic properties of doughs unless they associate with other molecules. Research is conducted on the effect of various conditions on gliadin-protein associations, particularly under conditions similar to those in bread doughs. Some gliadin components have been isolated and their properties are under preliminary investigation.

Basic studies on the types of bonding responsible for cohesiveness and related physical properties of gluten were concluded in contract research at the Midwest Research Institute in Kansas City, Missouri. They developed procedures for preparing freeze-dried crude gluten, purified gluten, and glutenin of high-quality in quantities of several hundred grams each, and they studied the viscoelastic properties of the preparations under various chemical conditions. When the water content of all the gluten systems was increased, the stress strain modulus decreased. Viscoelastic properties of crude gluten were not significantly changed by variations in salt concentration, urea concentration, or pH. Glutenin has a higher elastic modulus and

slower rate of stress decay than has crude gluten. Complete acetylation of free amino groups destroyed the cohesiveness of gluten, indicative of the role of hydrogen bonding in determining gluten's cohesive properties. Methylation of free carboxyl groups appeared to reduce the strength of crude gluten relatively more than that of purified gluten or glutenin. However, the effect might have been related to starch impurities in the crude gluten. Blocking of free sulfhydryl groups reduced cohesion of the purified gluten, but it did not affect the cohesion of crude gluten or glutenin.

Flour proteins, yeast, and other bread ingredients are rich sources of enzymes. During dough mixing and fermentation, enzymes become increasingly active, causing chemical breakdowns and recombinations that affect bread quality. Protein-splitting enzymes of wheat flour, for example, were found to hydrolyze the alpha$_S$-casein component of nonfat milk solids. Data from experiments at different pH levels indicate that the same system(s) of flour proteinases are active on hemoglobin and alpha$_S$-casein substrates. When alpha$_S$-casein was reacted with wheat flour, three characteristic hydrolysis products resulted. They were electrophoretically identical with reaction products obtained when a protease concentrate from flour was used instead of whole flour. The isolation of these specific protein fractions is further evidence that the protein-splitting activity of wheat flour is of a restricted and specific nature. The splitting of milk protein by wheat flour enzymes may be connected with the differential tolerance that flours have for added nonfat milk solids in dough formulas for continuous-mix bread making.

Two major types of protein-splitting enzymes of flour are distinguishable by their solubility characteristics. They differ in their activity dependence on pH and ease of denaturation. One has a maximum activity at pH 4.5 and is inactivated if sulfhydryl groups are blocked by use of a chemical reagent. This enzyme is relatively insoluble in water. Earlier attempts to extract it from flour generally led to its inactivation. We were able to extract much of the proteinase from flour using dilute acetic acid. With four sequential extractions, some 70% of the total protein-splitting activity was obtained, and most of the rest of the original activity could be accounted for in the residue. The extraction of this enzyme without loss of activity opens the door to its further purification and characterization so that studies can be conducted to determine its role in flour protein interactions. The other enzyme appears to have a maximum activity at about pH 3.8. It is also being characterized.

Basic chemical information on the function of sulfhydryl groups in protein-splitting enzymes from wheat is being developed at the University of Poznan in Poland under a P.L. 480 research grant. To determine more specifically the dependence upon sulfhydryl groups for protein-splitting properties, one of the wheat enzymes was isolated and purified and its homogeneity established by paper electrophoresis. This enzyme (designated protease A) contains both reactive and masked sulfhydryl groups. Its amino acid composition is unusual in that the proportion of cystine is high. Gluten, when incubated

with the isolated wheat proteases, is relatively resistant to their hydrolytic action, compared with other standard proteins such as hemoglobin.

2. Dough Rheology. Control of the mixing processes in large bakeries depends upon empirical measurements of resistance to mixing and extensibility of mixed doughs. In order to obtain fundamental data that will be useful in developing more sophisticated equipment and procedures for the testing of flour for bread-making quality and for making significant improvements in flour usage, we have initiated a series of basic investigations. In contract research recently started at Stanford Research Institute in Menlo Park, rheological properties (physical factors concerned with the plastic flow of materials) of wheat flour dough are being studied to determine relationships between molecular organization in doughs and their baking characteristics. Additional basic investigations are being supported by Public Law 480 grants to the Rheological Laboratory of the Israel Institute of Technology at Haifa and to the Bread Research Institute of Australia in North Ryde, New South Wales. Two different approaches are being taken under these research grants, but both are directed toward providing better measurements of the viscoelastic properties of flour doughs.

The project in Israel provides rapid measurements of tensile stress-and-strain relationships, relaxation time, and elastic recovery behavior in dough specimens taken at intervals during a dough-mixing cycle. These very precise measurements revealed that during an extensograph run of about 20 seconds the apparent coefficient of viscosity of doughs decays in a dramatic way. A 20-second extension of a molded piece of dough reduced its viscosity to less than 1/5000. Without the extensograph treatment, the viscosity of molded dough held up to 45 minutes. Salt had little or no influence on the viscous behavior of dough, and hence the pronounced influence of salt on extensograph values must be ascribed to something other than an effect on viscosity.

Studies such as these are complicated by the intricate interrelations of flow properties of a dough and the continuous changes, both physical and chemical, that occur in raw material used over a number of weeks or months. Stored flour changes, so that a series of experiments may reflect changes in the raw material in the system. Considerable effort must be taken to be certain that experiments are not unduly affected.

The scientists at the Bread Research Institute of Australia are using equipment designed to measure viscoelastic properties by applying a dynamic sinusoidal loading in contrast to the measurements employing a static loading in the Israel project. The two projects thus complement one another. The research grant in Australia is recent, and only preliminary findings have been reported thus far. They indicate that the equipment as now designed will be satisfactory over a low range in the frequency of stress application. The possibility of extending the usable range of the equipment by modifying the equipment design will be investigated.

3. Analysis of Protein and Related Components. Because the development of bread dough and the structure of wheat bread depends on the particular

proteins that wheat contains, we seek basic knowledge of these proteins in order to control raw materials and improve products and processing technologies. To understand wheat proteins, the individual proteins must be separated and their chemical composition and functional properties studied. Classical separation of proteins is based on differences in their solubilities in various liquids and solutions. By the most recent laboratory techniques, soluble proteins are studied by gel electrophoresis, which involves differences in molecular size and charge. Once separated by such techniques as gel filtration or preparative electrophoresis, the proteins are characterized by molecular weight, amino acid composition, and biological activity, particularly their functions as enzymes.

An important property of all proteins is related to their antigen-antibody reaction in the blood stream of animals. Specific antibodies develop when a foreign protein enters the blood. Upon subsequent exposure to the specific protein, these antibodies react, causing allergies or anaphylactic shock in animals or, in the laboratory, precipitation of blood components. This antigenicity, which can serve to characterize proteins, is the basis of the immunoelectrophoresis technique innovated by Dr. Grabar at the Pasteur Institute in Paris. With grant funds under P.L. 480, Dr. Grabar is continuing his research on cereal proteins. He concluded that an antigenic identity exists between alpha-amylases of barley, rye, and wheat, and that partial identity exists between these alpha-amylases and those of maize and oats, even though the latter are of different electrophoretic mobility. The beta-amylases of barley have antigenic identity, even though their molecular sizes differ. The presence of identical antigenic sites on protein molecules from different cereal sources may provide useful clues for ultimate studies of the molecular architecture of such proteins.

Immunochemical studies of soluble proteins from flour are also conducted in-house, including comparisons of hard red spring and durum wheat varieties. The results obtained so far by conventional and immunochemical gel electrophoresis techniques indicate that no clear qualitative differences exist between the soluble proteins of durum and hard red spring wheats. Any differences in processing quality related to the soluble proteins would thus appear to be an influence of quantity of such components, not kind. Confirmation of these results depends somewhat upon obtaining larger quantities of isolated soluble protein components. Work has been initiated on such a separation by use of continuous preparative electrophoresis on free-film equipment.

In contract research at Washington State University in Pullman, Washington, scientists are developing techniques necessary to use radiotracers in wheat proteins in order to detect the participation of individual proteins in the changes that occur during mixing and baking of bread. Wheats were grown in chambers under conditions whereby radioactive carbon atoms were incorporated in the various proteins of the wheat produced. Physical and chemical methods will be used to follow changes in these proteins after the wheats are milled to flour and the flour mixed into dough and made into bread in subsequent

studies. Slight differences in growth chamber conditions and field conditions were encountered that appear to be influencing the protein composition of wheat being grown in this investigation. This observation will be scrutinized with great interest because it implies a control of wheat protein composition by cultural conditions of production.

Basic studies on the solubility of wheat gluten proteins in aqueous systems were initiated with a P.L. 480 grant to the National Center for Scientific Research at Montpellier in France. Although gluten is considered insoluble in water, prolonged extraction after removal of the easily soluble protein provides additional quantities of a gliadin-like protein material. Attempts will be made to determine any correlation between quantities of this wheat component and baking quality of flour.

Another attempt to study insoluble gluten involves application of ultrasonic vibrations to suspensions of wheat gluten for the purpose of fractionating and modifying individual constituents of the protein for further identification. This work, supported by a P.L. 480 grant, is being conducted at the National Institute for Agronomic Research in Paris, France. Greater changes were induced by ultrasonic vibrations in gluten dispersed in acetic acid solutions than could be obtained using undispersed gum gluten. Solubilized material is being fractionated on dextran columns and the fractions are characterized by electrophoresis methods. Thin-layer chromatography of component amino acids is also being used to identify separated proteins and protein fragments.

At St. Albans, England, a P.L. 480 grant has been made to the Research Association of British Flour Millers, where protein is being solubilized by a novel method. Reactions with metallic and acidic ions, such as cupric and sulfite, were previously found to solubilize wool and feather proteins. Similar reactions are being adapted to study the less soluble protein components of wheat. Gluten solubilization has been obtained by use of sulfite ions in solution but good characterization of components by gel electrophoresis has not been achieved. Behavior on Sephadex columns is being interpreted in order to characterize different components of the gluten solubilized by sulfite treatment.

The interactions of proteins with ions and small molecules often play a basic role in their function. Such interactions can be examined by measuring molecular volume changes and such measurements are under investigation using the interactions of calcium and magnesium ions and adenosine triphosphate as a model system. In other studies on thermal decomposition of proteins, mass spectrometric data indicate that gliadin and glutenin undergo little degradation in the dry state up to high temperatures (170° C.). At higher temperatures, sulfur-containing amino acids break down first, with further breakdown and charring at temperatures above 200° C.

Because polysaccharide components of wheat are known to be related in some way to the baking quality of flours, we are studying the polysaccharides and

the related glycoproteins of wheat flour. Procedures for extracting water-insoluble pentosans from wheat flour were developed and a number of fractions separated by chromatographic procedures with various solvent combinations. While some separated fractions differ in chemical reactivity when hydrolyzed, all fractions examined so far contain the same groups of 5-carbon and 6-carbon sugars after hydrolysis. Unlike water-soluble pentosans that have been studied elsewhere, these water-insoluble ones have not contained galactose, and they have a very low protein content. We have found that water-insoluble pentosans can be solubilized in fluorinated organic solvents without the side reactions that occur between pentosans and some organic solvents.

The pentosans that exist in combination with proteins as glycoproteins are involved in one of the oxidation phenomena noted in wheat components. Such pentosans form gels when they are oxidized with bromate and iodate. The changes that occur during oxidation of pentosan glycoproteins are being studied at the Swiss Federal Institute of Technology at Zurich under a recent P.L. 480 grant.

At the University of Bologna in Italy a basic investigation of nitrogenous components of wheat germ is being supported by P.L. 480 funds. Wheat germ contains nitrogenous components of high biological value and a rational utilization of wheat germ in connection with nutrition, whether for food or feed, depends upon a better knowledge of these components than we now have. These studies have revealed the presence in wheat germ of appreciable quantities of protamines, several of which had not previously been found in plant sources. These include compounds found in putrefying animal flesh and others that are involved in animal reproduction.

4. Maturation of Wheat Flour for Bread Making. The addition of oxidative improvers to wheat flours in modern milling and baking eliminates the lengthy natural period of maturation required for some types of flour, particularly those made from hard red winter wheat. Effective treatments have been developed that are widely regarded as safe. Even so, regulations of continental European countries prohibit the treatment of food materials with almost any chemical agent, so that flour-maturing agents common in America and the United Kingdom are not allowed. These regulations hinder the development of commercial markets for our most abundant wheats. Therefore, our research program includes a number of projects aimed at achieving a better understanding of the maturation process with the long-range objective of finding acceptable alternative methods for maturing wheat flour.

Evidence is accumulating that flour lipids are involved in wheat flour maturation. We have found that the ratio of non-polar to polar lipid is higher in the hard red spring wheat flour, which requires little or no maturation, than in hard red winter wheat flour, which requires maturation or oxidative improvement. Treatment of the flour with oxidants affects the ratio. Preliminary data indicate that the ratio is slightly increased by bleaching with benzoyl peroxide or acetone peroxide. These studies will continue.

During prolonged mixing of dough, its sulfhydryl content increased if oxidation was prevented by blanketing the mixer system with nitrogen. In the first five minutes of mixing, sulfhydryl content decreased rapidly, then returned rapidly to the original level and increased with subsequent mixing. If lipids were extracted from the flour with carbon tetrachloride, the initial rapid loss of sulfhydryl during dough mixing was eliminated. This observation implicates lipids, probably oxygenated lipids, in some way with the changes in sulfhydryl.

Other investigators have reported that bromate and iodate oxidative improvers are consumed at a steady rate during dough mixing, but at a much slower rate when the dough is resting. They proposed that the effect of mixing was to diffuse the oxidants and thus speed the rate of reaction with sulfhydryl groups. Our findings support a different concept: that mixing releases sulfhydryl groups making them available to react with the oxidants. We attribute the initial loss of sulfhydryl groups (when dough was blanketed with nitrogen) to the oxidation of the few reactive groups originally present in the flour. Subsequent reactions depend upon the release of sulfhydryl groups by mixing.

Grant research is in progress at the University of Wisconsin to determine the important oxidative enzymes in wheat flour and the nature, extent, and conditions of their activity in dough systems. Lipoxidase, catalase, and cytochrome oxidase activities were observed in extracts of whole wheat and endosperm, and ascorbic acid oxidase and pyrogallol oxidase in whole wheat but not in endosperm. Much higher levels of enzyme activity were found in mill fractions, break shorts, reduction shorts, bran, and red dog than in flour. Of the enzymes studied, lipoxidase and catalase were by far the most active in the one hard red winter wheat variety studied so far. Other varieties will be examined, and quantitative estimates of enzyme activity will be made.

Studies of the coenzyme role of riboflavin of wheat endosperm are conducted under P.L. 480 at the Agricultural Higher School in Poznan, Poland. Flavin nucleotides are the indispensable components of several enzymes involved in biological oxidation. The amount and form of flavins in wheat flour from the various market classes of wheat and any coenzyme function of commercial importance are being determined. Appropriate methods are being developed for extraction of both loosely bound and tightly bound flavins from wheat flour for accurate quantitative determination of the flavins. The general procedure for flavin concentration by phenol had to be rejected as inappropriate for flour extracts, but concentration by cation absorption appears to be of value. Development of analytical procedures and selected instrumentation were tested and advanced to a routine basis.

5. Lipids and Lipoproteins. Closely related to the studies of wheat flour maturation are compositional investigation of lipids and lipoproteins. Benzene-soluble material from wheat flour was precipitated with acetone to give a material that contains lipoprotein, and the nature of the chemical

binding between the lipid and the protein was investigated. Since phospholipids are good metal chelaters, the first approach was to study the metal binding of phospholipids. In model compounds made by removal of fatty acid radical groups from two typical phospholipids, phosphatidyl serine and triphosphoinositide, the stability of metal complexes was determined by pH titration in aqueous micellar dispersions. Stability constants for the intact lipid metal complexes were 10 to 100 times greater than those of deacylated models. This greater stability is attributed to electrostatic properties leading to micellar aggregation in aqueous solvents. Surface charge characteristics greatly favor complex formation. Our hypothesis that lipids and proteins can interact through mixed metal chelate formation is supported by these data.

In contract research conducted at Kansas State University, lipids were extracted from flour milled from 16 wheat varieties (including hard red winter, hard red spring, soft red winter, durum, and soft white club wheats) for comparison of lipid composition with baking characteristics. Differences in lipid composition were relatively small, and the relationship of the lipids to baking characteristics of the flours was not apparent. These flours represent substantial quantitative differences in protein content and a wide range of responsiveness to oxidative improvers as well as substantial differences in baking quality. Interrelationships of these characteristics have not yet been established.

A basic study, supported by a P.L. 480 grant, of the lipids of whole wheat, ground wheat flour, bran, and other milling fractions is being conducted at the French School of Milling in Paris. The fatty acid composition of wheat lipids is being compared for a number of market classes of United States wheats. In addition, the effects of wheat lipid components on dough mixing characteristics are being investigated. Total lipids were extracted from flours and were fractionated. Defatting increased the Alveograph resistance to extension of doughs but after reaching its maximum, the resistance decreased faster than for normal doughs. If oxidation was allowed, the decrease in resistance was even faster. If sulfhydryl groups were blocked and prevented from reacting in the dough, there was no rapid loss of resistance. Reincorporation of unsaturated (oxidizable) fatty acids increased the resistance. These studies are providing important clues to the mystery of bread dough quality involving protein interactions, lipids, and oxidative improvers.

Flours and glutens from 10 United States wheats, including club, soft, hard, and durum samples, are being studied under a Public Law 480 grant to the National Institute of Agronomic Research in Paris, for their content and type of phosphorus compounds related to lipid and protein composition. The phosphorus in albumin-type proteins is generally of a phytic nature, whereas that in globulins is in the nucleic acid form, chiefly desoxyribonucleic acid. Hard spring and durum flours contain more phytic acid phosphorus than does hard winter wheat flour, and soft wheats contain less phospholipidic phosphorus and less gluten ribonucleic acid phoshporus than do hard wheats

and durums. Methods of analysis are still being refined in this research and it remains to be seen whether measurements of phosphorus components will be useful to predetermine baking quality of various wheat flours.

Under a P.L. 480 grant, the National Institute for Agronomic Research in Paris, France, is investigating the lipase activity of wheat as a function of water vapor tension, partial pressure of oxygen, and temperature. Methods are being developed for extracting and isolating lipase of wheat and determining fatty acids released by lipase action in wheat held at very low moisture contents. The lipase activity of wheat at various stages of germination and growth has also been studied. Lipase activity appeared to be closely correlated with the growth and size of wheat seedlings. Temperature conditions that retarded or enhanced plant growth also retarded or enhanced the lipase activity.

6. Flavor. Fresh-bread flavor is an important factor in enhancing bread consumption. The instability of fresh-bread flavor remains a technical challenge to research. Basic investigations are revealing the chemistry associated with bread flavor. Now if we can learn specifically what components are responsible for flavor and how to measure them, we can provide the cereal chemist and baker with tools for flavor enhancement and flavor stabilization. An aroma concentrate resembling bread flavor was made from a brew of normal bread preferment ingredients except that flour was omitted. An extract was taken after four hours of fermentation and concentrated. This concentrate had a vaguely reminiscent odor of bread, but when it was heated in air at 210° F. it produced a very definite aroma of baking bread. Although the extract as obtained is a complex acidic liquid, neutralization of the acid did not affect aroma characteristics. Gas chromatography analysis indicated that the concentrate extracted from preferments had many of the same components as did condensates of baking oven vapors, except that the oven vapors had several very high-boiling components not present in significant quantity in the preferment extract.

Causes of flavor instability of wheat bulgur products are also being studied by gas chromatography. In the early stages of storage deterioration of wheat bulgur shelter wafers, we have detected hydrocarbons in the vapors from the wafers. To augment the study of wheat products, we use model systems, including fatty acid methyl esters. Of the esters treated to promote autoxidation, only methyl linoleate and methyl linolenate produced hydrocarbons. Methyl laurate, methyl oleate, and methyl 11-undecenoate did not produce hydrocarbons over an eight-week period of autoxidation. Thus, it appears that two or more double bonds are necessary in a fatty acid ester for hydrocarbon formation to occur during the initial stages of autoxidation. Oxygen uptake by methyl linoleate suspended in glass wool in an oxygen atmosphere was relatively slow for about a week and then very rapid for about 10 days. It decreased slowly thereafter. Three moles of oxygen gas were consumed per mole of methyl linoleate after 30 days. The high ratio of oxygen consumption strongly suggests that secondary oxidations occur. Gas chromatographic analyses of headspace gases above ground bulgur and ground puffed bulgur stored

under oxygen or nitrogen at four temperatures will be compared with evaluations by odor panels to determine whether any of these oxidized compounds would be useful as advance indicators of rancidification.

7. Pigments of Wheat Bran and Aleurone. Contract research has been initiated at Oregon State University to determine the nature of the substances in bran and aleurone tissue that are responsible for coloration of wheat grain. The inner true bran layers of wheat contain most of the coloring matter of the kernel. Basic studies of bran pigments should be helpful in developing decolorizing procedures for use in whole kernel products. Pigments and phenolic compounds closely associated with pigments have been extracted from bran by sequential leaching with organic solvents and water, and individual compounds separated by paper chromatography after preliminary separations in solvents. Large-scale extractions were made to provide material for further study from genetically pure strains of three varieties of red wheat.

B. New and Improved Food Products and Processing Technology

1. Bulgur and Related Wheat Products. Recent developments of export markets for bulgur, a parboiled dried wheat used since ancient times in the Near East, have been a major accomplishment in which Department utilization research scientists have cooperated with private industry and grower-sponsored marketing associations. Prior to 1961, there was no significant export market for bulgur. After a trial shipment in 1962 of 60 million pounds for welfare distribution in 27 foreign countries, bulgur exports have grown to supply markets for more than 8 million bushels of wheat per year. Most of this export depends on donations of government surplus and concessional sales, but the product has been well received in many places and efforts are being made to develop a commercial market.

In some markets, particularly in Hong Kong, resistance to the use of bulgur appears to be based on the dark color and high bran content of red wheat bulgur. To produce a quick-cooking, light-colored, bland-flavored product, even from hard red wheat, we have developed a lye peeling method to remove the bran. We call this product WURLD wheat. Pilot-plant operations have been developed to study the process and produce a product for demonstration purposes. About 2500 pounds of WURLD wheat from hard red winter wheat were shipped to Hong Kong for pilot acceptance tests by voluntary welfare agencies there. The preliminary test was gratifying, and requests have been made for more WURLD wheat to evaluate its potential in commercial markets as well as its use for welfare purposes.

WURLD wheat is prepared by precooking wheat, treating it with concentrated alkali to soften the bran adhesion, quenching the alkali in water, scouring the bran by turbulent water flow, separating the peeled wheat from the bran, and neutralizing any residual alkali with acetic acid. After drying, the kernels can be cracked or used whole.

The storage stability of WURLD wheat prepared from white club, hard red spring, and hard red winter varieties was evaluated. After six to eight

months' storage, differences in stability between bulgur and WURLD wheat were small and of no practical significance.

In addition to wheat, barley and other cereals can be peeled down to the aleurone layer by the WURLD process. In this way the loss of material (such as the ends of pearled barley) can be avoided.

A systematic study of the influence of several processing variables on quality of bulgur has been initiated. Soaking time and temperature, steaming time and temperature, and drying conditions are being evaluated as to their effects on the hardness of bulgur, its cooking time, and its expansion by hot-air puffing.

To develop a series of flavorful food products, research has been initiated on the effects of processing variables in the malting of wheat. Temperature and humidity during germination and toasting can be varied to produce a range of flavors and textures that should be useful in developing new food products and richly-flavored flour for blending purposes. In-house research will be supported by contract research recently initiated at the University of Wisconsin on changes in protein and other major nutrients of wheat during malting.

2. Food from Wheat Fractions. In milling wheat into flour, nearly 30% of the kernel becomes byproduct milling fractions, generally used as livestock feed. About two million tons of wheat feed, midlings, shorts, and red dog flour, and about an equal amount of bran are used for this purpose each year in the United States. These mill byproducts contain higher levels of protein than does flour or the starting grain, and the protein is of greater biological value. Research is conducted with the objective of using wheat fractions in new high-protein food products. Substantially all the protein is extractable from finely ground bran at pH 12.5, but the recovery decreases as the pH is lowered. However, only about three-quarters of the nitrogenous compounds can be precipitated from the solutions after extracting; the remainder is non-protein. Treating the bran with cellulose-splitting enzymes or hydrogen peroxide did not improve quality of the protein extracted. Protein recovered from bran by alkali extraction and acid precipitation has a dark brown color, poor stability to rancidification, and a strong branny odor and taste. Modifications in the recovery process will be sought to improve the quality of product.

An improved process was advanced for preparing a bland, milk-like nutritious drink from wheat or wheat fractions high in proteins. After acid-pepsin digestion of flour, prime starch can be separated from other flour components. The material other than prime starch is then heated to bring about gelatinization and partial hydrolysis of the starch fraction it contains. The neutralized and cooled product is a protein-enriched, bland liquid. Viscosity and protein content can be adjusted to those of milk by adding dextrins produced from the prime starch fraction. Drum drying of this milk-like liquid produces a powder that can be easily dispersed in cold water. It is relatively stable and retains most of the nutritional value of the starting wheat protein.

This development of a milk-like product is based, to a considerable extent, on preliminary studies of enzyme digestion of wheat protein conducted under contract at Purdue University. The work at Purdue was concluded after the testing of several procedures to improve the dispersibility and the stability of the dispersion of wheat proteins. Methods examined include deamidation with acid, hydrolysis with protein-splitting enzymes, and chemically induced splitting of disulfide bonds.

3. Emergency Food Supply. Foods suitable for stocking fallout shelters are being developed and evaluated with funds transferred to Agriculture by the Department of Defense. The level of support for this project was substantially reduced from previous years. In-house research includes basic studies on oxidative deterioration and stability of wheat food products (see paragraph 1-A-6). The contract evaluation of stability of bulgur wafers and new food adjuncts for a wafer-based ration continues at Oregon State University. Taste panel evaluations of bulgur wafers after 22 months of storage and chemical analyses after 16 months stored at three temperatures indicate that product stability at this stage is satisfactory even up to 100° F. Packing in nitrogen is preferable to packaging in air, and formulation with malt syrup is preferred to formulation with corn syrup. Differences in chemical tests do not, at this stage, show trends consistent enough to correlate with test panel evaluations. Such correlation is sought for use in developing a stockpile surveillance procedure.

4. Baking Quality of Flours. To provide improved testing procedures for baking quality of flours, to assist in development of improved varieties, and to check the accuracy of our test procedures, we are collaborating with the Hard Winter Wheat Quality Council in comparing values obtained from farinograms, mixograms, and pressure-meter measurements of diastatic activity from straight doughs made by a 3-hour fermentation procedure. Included in the tests this year were samples of 26 hard winter wheat flours from six states. A major function of the Council is to recommend release of new wheat varieties for commercial production. An important benefit to us resulting from this collaboration is the evaluation of our test procedures. Since our results have been comparable with those of most other testers, we believe we can use our methods with confidence to evaluate bread-baking performance of high-protein fractions obtained by air classification of hard red winter wheat flours and blends of high-protein fractions with low-protein flours.

We are currently testing low-protein fractions from air-classified flours for performance in cakes. Low-protein fractions from five different wheat flours were bleached with chlorine gas. The treatment produced a different pH in each flour. The cause of these differences in chlorine-induced pH changes has not yet been learned, but the pH change does not correlate with the relative surface area of the flour, the proportion of gluten in it, the proportion of low-buffered prime starch, or the ash content--four factors that have been mentioned by others as having an effect on the chlorine requirement for bleaching flour to a specific pH.

Continuous-mix bread-making processes, in general, tolerate less nonfat milk in formulation than do sponge and dough methods, and some flours affect this tolerance more than do others. Even though flour and milk present two very complex protein systems, we are making headway in that we can see an implication of an active protease in flour capable of splitting a specific casein fraction of milk. The amount and activity of the flour protein enzymes, which vary from one flour to another, may be responsible for the variations in milk-carrying capacity of a continuous mix dough. We will isolate the major protein components from both the flour and the nonfat dry milk to provide sufficient quantities to incorporate in doughs for tests to provide evidence of specific effects produced by different protein components. For continuing studies of the effects of milk protein on continuous-mix bread, mixing and baking procedures are being standardized by use of the Brabender Do-Corder.

The effect upon baking quality of variation in wheat flour lipids was investigated under a P.L. 480 grant to the British Baking Industries Research Association in Chorleywood, England. They studied the influence of variety, season, and environment on the composition and baking quality of flours from five wheats grown in the United States and two wheats grown in Great Britain in the crop years of 1959-1962. The varieties did not differ with respect to the number and types of lipids present, but the quantity of these lipids varied substantially with variety, season, region, and environment. Apparent correlations between analytical data and baking performance were not consistent from one season to the next. Baking tests demonstrated that fat in the presence of the optimum amount of an oxidizing agent can exert an improving effect on bread, and a pure synthetic saturated lipid had a similar effect.

Another P.L. 480 grant has been awarded to the same institution to study the structure of dough and baked products as a function of interactions between dough constituents, especially the interaction of lipids. This new investigation will build on the foundation of competent research developed in the first grant.

5. Carrying Capacity of Hard Red Winter Wheat. In Western European countries large amounts of wheat are grown that are too soft and of too low a protein content to make high-quality yeast-raised bread. To add strength and tolerance to doughs and give uniform baking qualities, these countries import wheat to mix with their locally-grown wheat, but some of them restrict the amount of imported wheat that may be used. The hard wheats differ in their carrying capacities, that is, the degree to which a given percentage of hard red wheat will improve the soft wheat. Flours from the flinty, vitreous hard wheats are granular and sharp compared with flours from soft wheat, and inadvertent classification may occur during or after blending. Furthermore, different types of hard wheats react differently when blended with soft wheat and mixed into dough for bread baking. In order to provide information that may expand markets for surplus United States hard winter wheats, we are investigating the carrying capacity of the hard U.S. winter wheats. Research on blending characteristics was initiated by contract at Kansas State University with

samples of soft wheats procured from Germany, England, Sweden, The Netherlands, Austria, France, and Belgium and samples of hard red winter and spring wheats from the United States and Canada. Flours milled from these wheats will be blended to provide a range in protein content and evaluated for baking and dough-mixing properties.

6. Nutritive Value of Processed Wheat. In developing wheat food products, attention is given to the highest possible degree of nutrient retention. In WURLD wheat, which was developed to overcome objections to the color and fiber content of bulgur, the bran and germ normally present in bulgur are removed by an alkaline treatment that reduces the crude fiber content and lightens the color of the product. The process is designed to retain as much of the aleurone tissue as possible because of its high nutritive value. Average levels of thiamine, riboflavin, vitamin B_6 and folic acid in WURLD wheat are somewhat lower than in bulgur made from the same wheat, but more than half of the amount originally contained in the wheat is retained. Even greater proportions of niacin, choline, and pantothenic acid are retained. Protein efficiency ratios for WURLD wheat are only slightly less than for bulgur. Although these values represent lower levels of nutrients than in whole ground wheat, they exceed by far those in white flour.

Rapid chemical methods for assay of the biological value of wheat proteins during processing are being developed under P.L. 480 at Cambridge University in England. A chemical method or a combination of chemical methods that will correlate with rat- and chick-feeding tests for availability of protein in wheat foods is sought. The success of this work would simplify and reduce the cost of biological evaluations and should provide, also, a substantial increase in the amount of data available for use in improving food products. A chemical analysis for "total tryptophane" has been partially successful as an indication of protein efficiency ratio for some products, but not for wheat flour or wheat products. Several attempts to reduce hydrolysis losses in determining available lysine were made, but none of the approaches used was completely successful. As a result, chick-feeding tests are still being used to determine effects of processing treatments on nutritional quality of wheat products.

When wheat is steeped in hot water, its protein efficiency ratio appears to be increased but the effect later proved to be due to more efficient use of carbohydrate present. Such steeping is a preliminary step in both bulgur and WURLD wheat production.

7. Microbiology of Wheat Food Products. Relative freedom from microbial contamination of flour to be used in precooked frozen foods, baby foods, and certain canned products is an important safety factor for such foods. Under contract research, the American Institute of Baking in Chicago is determining the nature and extent of microbial contamination in wheat flour and studying means to reduce or eliminate it. Flour inoculated with four types of contaminating micro-organisms at the level of one million organisms per gram

has been treated with propylene oxide. The treatment destroyed the most resistant of the micro-organisms, B. subtilis, even in its stabilized spore form. No significant damage to flour properties was found, but the economics of the treatment are yet to be worked out.

Survival patterns of flour-introduced microbial contaminants were determined in frozen soups and pie doughs. E. coli and A. flavus contaminants were destroyed in the food processing operation, or they survived for less than a week in frozen storage. S. aureus and B. subtilis were virtually unchanged as a result of the freezing and storage treatments.

C. New and Improved Feeds and Feed Processing Technology

1. Improved Feeds from Wheat and Wheat Fractions. Wheat milling byproducts contain important nutrients at levels of concentration higher than in milled flour, but because of general variability in quality and condition, they cannot compete with protein, carbohydrate, and other nutrients from other sources for use in mixed feeds. The unrealized feed values in mill byproducts adversely affect milling profits and are reflected in higher flour prices as well as in pressure to reduce wheat prices to growers. Research is being conducted on enzymic release of carbohydrates from cellulosic components of mill run. Preliminary results of using salivary amylase were promising. Bacterial and fungal amylases did not work well. Research is underway to provide better analytical methods for estimating biological value of mill run fractions.

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AREA NO. 2. RICE--PROCESSING AND PRODUCTS--WESTERN LABORATORY

Problem. The productive capacity of U.S. rice growers has increased faster than domestic and export consumption in recent years to cause imposition of acreage limitations and restrictions in the income potential for growers and the U.S. economy. New and diverse food products from rice that are easy to prepare, have greater texture and flavor appeal, and are economical to manufacture are needed to increase the total consumption of rice both here and abroad. Likewise needed are drastically improved milling methods to increase economic returns to growers and millers from the limited production allowed. Detailed knowledge of chemical composition and physical properties, as related to processing, is needed to guide the developments in milling, processing, and products required to achieve expanded markets.

USDA AND COOPERATIVE PROGRAM

In the Western Utilization Research and Development Division, basic and applied research on rice is conducted at Albany, California. Basic studies involve chemical, physical, and biochemical investigations of rice proteins and carbohydrates and changes undergone by these constituents during processing. Process development is underway on debranning of brown rice by lye-peeling, parboiling of brown and undermilled rice, new methods to produce quick-cooking forms having better flavor and texture, and on conversion of high-protein flours into beverage products especially suitable for infant feeding overseas. New gloss-producing agents to replace talc for coated rice are in final stages of commercial-scale testing.

The Federal program of research in this area totals 2.9 professional man-years. Of this number 1.1 are assigned to chemical composition and physical properties; 1.8 to new and improved food products and processing technology.

PROGRAM OF STATE EXPERIMENT STATIONS

Rice research in progress at the State stations involves evaluation of new rice selections and varieties for agronomic and milling quality. Emphasis is placed upon developing and applying rapid, simple testing procedures useful for screening selections. Much of this work is carried out in cooperation with the Regional Rice Quality Laboratory. Laboratory methods for determining the quality of milled rice are sought for use in establishing objective standards for measurement of the quality of cooked rice. Cultural practices that may affect the quality, drying properties and storage stability of rice are evaluated to determine their influence upon processing characteristics and product quality.

Basic composition studies relate to the quantity and quality of the proteins, lipids and starch fractions and to their distribution within the kernels.

Occurrence of mycotoxins in rice is being studied. Attempts to develop methods for the prevention and control of mycotoxin elaboration in rice during processing and marketing continue.

Product research involves development of effective ways to use rice in quantity food service. Another study seeks ways to make more efficient use of rice proteins through study of the supplementary value of high-protein foods derived from rice and its by-products. The biological value of the proteins of rice, when used with multipurpose food is being investigated. This research is especially timely in view of the potential for use of rice in the diets of developing countries.

The influence of drying methods used on rough rice on the processing characteristics of rice is under study. Other variables such as maturity and variety are also studied.

The total State scientific effort devoted to utilization of rice is 1.9 professional man years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Rice Proteins. Rice protein has a high biological value relative to other cereal proteins. We are investigating the amino acid composition of two globulin fractions. One fraction precipitates at pH 4, contains two components, and has almost no lysine or histidine. The other, soluble at pH 7, has five or six components and is rich in sulfur-containing amino acids. We are separating components of these fractions for further study.

About 0.4 to 0.7% of rice protein has been extracted as prolamine, which is distinctly different from the globulins. It contained some 20% of non-protein material. The entire prolamine fraction has been divided into 7 components by electrophoresis.

B. New and Improved Food Products and Processing Technology

1. Improved Rice Products. Deep milling of white rice to produce a flour having double the protein content of the starting, normally-milled kernel was reported a year ago. Development of this high-protein rice flour is being investigated at both the Southern and the Western Utilization Research Divisions. At the Western Division we are comparing amounts and types of proteins in the flour with those in the original rice and working on procedures using commercially available equipment for deep milling to increase the protein content of the rice flour. A single-pass abrasive milling with equipment similar to certain types available commercially, yielded 3-4% of high-protein flour. Breakage was 2% or less. Second heads (large broken kernels) provided somewhat less than a 2-fold protein increase. Air

classification of defatted rice polish and bran provided some concentration of protein in the product fractions.

Rice flour containing 15-1/2% protein has been shipped to the United Nations Childrens' Fund for protein evaluation by animal feeding tests and for accompanying analyses. Because rice is the common man's food in many areas of the world where protein deficiency is a major nutritional problem for young children, the deep milling of rice to provide a protein-rich food is potentially a very significant development of utilization research.

A high-gloss rice is preferred in the Caribbean markets where much California-grown rice is sold. High gloss is customarily achieved by coating the milled rice with glucose and talc, but since talc is not metabolized, regulatory agencies are concerned about its use. We have been testing a number of alternative materials for use in the sheen-coating. Limited commercial trials with calcium citrate or calcium acetate in place of the talc appear promising. The acetate gave less sheen than does talc, but the citrate produced a sheen nearly like that from talc. Taste panels were unable to detect any flavor difference related to the citrate coating.

Color formation during processing of parboiled rice is generally believed to be due to the browning reaction, but pigment from the hull and bran may also contribute substantially to the overall color. In an investigation of the effects of processing variables in parboiling brown or undermilled rice, we found that increased pressure during cooking yielded parboiled rice with a lower percentage of broken kernels, but color of the rice was darker when higher cooking pressures or longer cooking times were used.

Brown rice can be peeled down to the aleurone layer by the WURLD process. Such rice is absolutely white. Even in a raw form it can be steamed in 20 minutes for eating. It should have a higher retention of the protein, vitamins, and other nutrients known to be located in the aleurone layer.

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AREA NO. 3. FORAGES AND FEED-- PROCESSING AND PRODUCTS

Problem. The demand for livestock in the United States will increase 45% by 1975. Forage crops constitute the major feedstuff for ruminant animals. In addition, there is an increasing demand for processed forages in European and Asiatic export markets. Fresh forage crops are the richest natural source of many nutrients for farm animals. Forages, however, are preserved so inefficiently by haymaking and ensiling that 10 to 50% of the dry weight, and much larger fractions of the most valuable nutrients, are lost before the animals eat them. Dehydration is now the only practical means of producing high nutritional value products in a form usable in manufactured feeds and supplements. Poultry and swine producers are aware of the value of dehydrated forage, but restrict their consumption because of high fiber and growth-inhibitor content. There is evidence to show that certain unidentified growth factors are at least partially lost during the dehydration process as presently carried out. The livestock breeder needs forage products tailored to specific animals, and the forage producer must adapt to his needs to sell.

Basic and applied utilization research are necessary to produce: (1) high protein, low-fiber feeds rich in unidentified growth factors designed for use by non-ruminant animals; (2) fiber products which have been cheaply treated to make them easily digestible for ruminants; (3) growth stimulating supplements for ruminants based on the biologically active fiber digestion factors and growth-promoting factors in forage. New products should be adaptable to mechanical feeding. Improved uses will encourage farmers to put high-value land now producing surplus crops into forages.

USDA AND COOPERATIVE PROGRAM

Current research in the Western Utilization Research and Development Division includes both basic and applied studies on all forages used or potentially usable for off-the-farm processing. The research is conducted at the Division headquarters at Albany, California; under contract at Berkeley, California; Lincoln, Nebraska and Athens, Georgia; and under the P.L. 480 grant programs in Scotland and Italy. Basic compositional studies deal with the potent estrogen, coumestrol (discovered by Department scientists), and other phenolic compounds present in forage legumes. The value of coumestrol-rich alfalfa as a growth stimulant for ruminants is being studied with financial support of the Department of Agriculture and Inspection of the State of Nebraska and the cooperation of ARS Farm Research and several experiment stations and commercial processors of forages. Also under study are other biologically active forage constituents (such as the chick-growth-promoting factor in forage juices and alfalfa saponins which depress chick growth), organic acids of alfalfa, and the mechanism of action of forage

antioxidants. Processing of forages by "wet" (juicing) and "dry" (turbo-milling and air classification) methods is being investigated.

The Federal program of research in this area totals 9.9 professional man-years, including one scientist whose salary is provided by the Department of Agriculture and Inspection, State of Nebraska, and contract research equivalent to 2.8 professional man-years per year. Of this number 4.7 are assigned to chemical composition and physical properties; and 5.2 to new and improved feeds and processing technology. In addition the Division sponsors, under P.L. 480, two research projects on forage composition.

PROGRAM OF STATE EXPERIMENT STATIONS

State stations conduct an extensive program of both basic and applied research on forage utilization. Much of the research is interdisciplinary and often involves several departments.

One major segment of the research effort is devoted to determining the chemical composition of forages and evaluation of the relationship between chemical composition of certain forages and their nutritive value for farm animals. Evaluation of the effects of certain agronomic, cultural, processing and handling practices on composition, palatability and nutritive value of forages receives much research attention. Fiber content and utilization of fiber by swine, cattle, sheep and poultry affect the value and use of forages. Methods of isolating and analyzing for fiber are being developed. Investigation of normal and abnormal rumen fermentation of forages is fundamental to maximum utilization.

Careful studies of specific constituents of forages are being undertaken. Determination of certain minor elements found in forages is important both from nutrition and toxicity standpoints. Protein content and quality merit special attention along with determination of amino acid values and unknown growth factors. Leaf organic acids and proteins are investigated in detail in an effort to increase our understanding of their biosynthesis and properties in relation to growth of forage plants.

Due to the economic importance of forages in animal feeds, development of means for evaluation of the nutritive quality of forages has become an important field of study. New and more accurate or rapid chemical procedures are being sought.

Development of forage handling and processing systems to minimize labor costs has led to increased research on forage processing methods. Fermentation characteristics of and animal response to forages which have been wilted, chopped, pelleted, ensiled or dehydrated are being determined. Small-scale ensiling systems are being used to evaluate various silage preservatives. Methods of dehydrating alfalfa are being studied and the economic feasibility of dehydration is being investigated.

The total research effort devoted to forage utilization is about 22.7 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Chemical Constituents of Forages. Research is conducted on phenolic components of alfalfa and clovers. Twelve phenolic compounds already have been isolated and fully characterized. Coumestrol was the first and was reported in previous years; the other compounds have structures similar to coumestrol. Biological activity of compounds of this type include effects like those of female hormones. Coumestrol was initially isolated by following estrogenic activity of alfalfa fractions (see paragraph 3-B-1). The estrogenic and other biological activity of these compounds will be under continuing study.

2. Interactions of Forage Antioxidants. Carotenes, vitamin E, xanthophylls, and other related substances, provide much of the biological value of alfalfa. These substances gradually deteriorate when exposed to oxygen. We are trying to understand natural antioxidant activity in alfalfa to guide us in developing products that will not deteriorate. Contract research is conducted at the University of California in Berkeley on separation of alfalfa lipids and the relationship of lipids to carotene oxidation in forage crops. Mixtures of galactolipids were isolated by thin-layer chromatography. Galactolipids made up half the total alfalfa lipids, and methodology for separating mono- from di-galactolipids was improved. Two-dimensional thin-layer chromatography separated 18 components, but further improvements are required to separate and quantitatively recover individual lipid components. The extreme instability of these compounds complicates their separation. Light-induced changes and oxidation may alter these lipids before they are separated.

Under a P.L. 480 grant, research at the Experiment Station for Practical Agriculture in Milano, Italy, is underway on non-tocopherol reducing substances in alfalfa to throw light on the natural antioxidant activity of alfalfa. To prevent oxidative changes during separations in laboratory research, synthetic antioxidants are used as oxygen acceptors. They protect, for instance, carotenoid double bonds from peroxidation. Alpha-tocopherol has natural antioxidant activity. Paper chromatography separated and detected alpha-tocopherol and other antioxidant substances. One of the other reducing substances is plastoquinone. Surveys of reducing substances were made with alfalfa at different stages of maturity. Alpha-tocopherol and other substances under investigation tend to increase in amount as the plant matures. The distribution of reducing compounds in various parts of the plant was also surveyed. The stem had the same general composition as the leaves, but with reducing substances in far lower concentration. In yellow leaves, one specific compound was particularly prevalent, but this compound was absent in the flowers; it exists only in the chloroplasts.

3. Structure of Alfalfa Polysaccharides. Apparently combination with indigestible lignin reduces the digestibility of alfalfa polysaccharides. Research is being conducted under P.L. 480 funds at Edinburgh University in Scotland to determine the nature of alfalfa polysaccharides and investigate enzyme systems that may be helpful in structural analysis of alfalfa polysaccharides and components associated with them. Enzymes, which were extracted from alfalfa seedlings, could hydrolyze still other carbohydrates. Polysaccharide fractions were isolated from separated leaves and stems and the main structural linkages were determined for the stem hemicellulose.

B. New and Improved Feeds and Processing Technologies

1. Coumestrol-Enriched Feeds. Tests on growth response of sheep to pure crystalline coumestrol were completed at the Oregon Experiment Station in cooperative studies. They confirmed the growth-promoting effect of coumestrol which had been previously found by feeding alfalfa meal rich in coumestrol. Lambs on a ration containing coumestrol used feed 11% more efficiently and gained weight 18% faster than did lambs on the basal ration. The Indiana Experiment Station and the Animal Husbandry Research Division of the Agricultural Research Service are cooperating in the evaluation of coumestrol as a growth promoter in large animal feeding trials, but no conclusions have been reached yet.

2. Improved Alfalfa Meal. Studies on the improvement of alfalfa products by wet and dry fractionation procedures are supported in part by the Nebraska Department of Agriculture which provides the salary of one scientist. In cooperation with the Dixon Dryer Company, Dixon, California, experimental equipment for combined water extraction and dehydration of forages was installed at their plant. Successful runs provide data for planning expanded studies during the 1965 crop season. Some of these experiments on dry processing will be conducted in Nebraska. A supply of concentrated water extract from alfalfa was obtained for chemical characterization and bioassay. A separation milling procedure for alfalfa has been developed and was tested in 1964 on 20 cuttings of three plots of alfalfa harvested at intervals of 26, 30, and 34 days. Each sample of each cutting was processed to separate stem from leaf and analyzed for proximate feed composition, carotenes, and xanthophylls. By separating leaf meal, plants can be allowed to mature further and yield higher tonnage per acre without sacrifice of feed quality. Large samples were retained for bioassay of xanthophyll availability. A preliminary experiment indicated that the amount of pigmentation produced in poultry is markedly improved by pelleting and regrinding alfalfa leaf fractions. Large amounts of two types of saponins were prepared from alfalfa for further bioassay. Dimethylsulfoxide was useful to dissolve difficult saponins for bioassay with chicks.

3. Products from Southeastern Grasses. Contract research was initiated at the University of Georgia to study two southeastern forage crops, coastal Bermuda grass and Pearl millet. Four cuttings of Bermuda grass were made to

obtain forage of different maturity levels and six cuttings of millet. From each, samples were prepared of fresh chopped forage, dehydrated chopped forage, and pelleted meal with different antioxidant and antioxidant synergist treatments for use in stability studies. Carotene, xanthophyll, and tocopherol composition will be studied throughout storage tests. For the 1964 growing season in the area tested, millet had higher protein and higher carotene concentration than coastal Bermuda grass. Seasonal changes may be important so several years' testing are required for conclusive evidence.

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AREA NO. 4. WOOL AND MOHAIR-- PROCESSING AND PRODUCTS

Problem. Traditional markets for wool and mohair have been lost to synthetic fibers because consumers prefer garments that hold their pleats and creases, resist shrinkage and wrinkling during washing, and dry quickly. Natural wool and mohair outclass the synthetics in tailorability, comfort in wear, appearance, and hand, but demand certain features now being exploited by the promoters of synthetics. Furthermore, some current processing damages, distorts, or weakens wool and mohair fibers and injures performance and appearance of fabric. We need processes that will modify natural fibers to give a range of comfortable and attractive fabrics that resist deterioration in processing and wear. Fabrics must be durably resistant to wear, wrinkling, pilling, abrasion, yellowing, soiling, felting and relaxation shrinkage, acid and alkali weakening, insects, and microorganisms. New markets would develop for new types of fabrics, woven and non-woven, for industrial and other uses, made with natural wools and with blends of wool with modified wools or other fibers. Wool could have a part of the new, rapidly developing market for stretch fabrics if we could practicably impart permanent stretch into wool yarn. Research toward such developments requires fundamental information on the chemical, physical, and structural nature of natural fibers and their modified products.

To sustain a stable sheep and wool industry in the United States, mills must be supplied with processing information on new and improved wool and mohair products. Synthetics have cut into wool markets because they are uniform in price and quality and because detailed processing information is available from producers.

USDA AND COOPERATIVE PROGRAM

The Western Utilization Research and Development Division conducts a broad basic and applied research program on wool and mohair to develop new and improved fibers and fabrics that can increase markets. Fundamental research seeks new facts on chemical and physical properties of natural fibers that may make wool and mohair fabrics more useful and valuable. We use such knowledge to try to modify fibers and fabrics so that they will resist degradation by heat, light, chemicals, staining, abrasion, and insects; wash easily; retain creases; shed wrinkles; and require little care. We seek practical processes for chemical and physical modification of wool and mohair fibers, yarns, fabrics, and felts into products that will increase wool and mohair utilization. In addition, Department scientists make every possible effort to bring research results to the industry through technical publications, public service patents, popular articles, TV and radio broadcasts, participation in growers' and processors' meetings, exhibits, mill visits and development trials, and conferences with visitors from the industry.

The Federal program is conducted at the Division headquarters at Albany, California, by contract in Durham, North Carolina and Washington, D.C., and by grant funds under P.L. 480 in India, West Germany, Sweden, England, and Finland.

The Federal program of research in this area totals 33.6 professional man-years, including contract research equivalent to approximately 3.1 professional man-years per year. Of this number 12.0 are assigned to chemical compositional and physical properties and 21.6 to new and improved textile products and processing technology. In addition, the Division sponsors research grants under Public Law 480 including five on basic studies and one on the application of research findings.

PROGRAM OF STATE EXPERIMENT STATIONS

Station research related to wool and mohair utilization is limited. One major effort is directed toward developing suitable objective measurements for determining the market value of grease fleeces. Information on the relationship between farm value of raw wool and textile mill value of sorted and scoured wool is being assessed to improve the bargaining position of the producer and to assist the breeder and rancher in developing wool with more desirable fiber characteristics. Work at three western state stations is progressing to obtain data on physical and chemical characteristics of grease wool and mohair. These data include combing performance, scouring losses, content of foreign matter, moisture, and wool grease as well as fiber information on variability of fineness, staple length, crimp, strength and elongation.

Additional studies are being pursued on the merits of different types of packaging materials for raw wool and the feasibility of baling graded fleeces. Since wool fabrics, largely used for outer garments, are subjected to weather elements (sun, dust, and rain), a study designed to determine the comparative resistance of outside weathering is evaluating the rate changes in physical and chemical properties of worsted textiles differing in fineness and crimp. An investigation of the role of protein level in sheep nutrition is directed to determine its influence on the fineness, quality, and yield of wool.

The total research effort devoted to wool and mohair utilization research is approximately 3.4 professional man years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Chemical and Molecular Properties. Research is conducted to define the fundamental chemical properties of wool to support development of useful processes. In the course of this research, improvements in research methods are developed. Our publications over the past several years include original

contributions in optical and electron microscopy, light scattering photometry, ultracentrifugation, theory of light scattering, electrokinetics, infrared absorption, and measurement techniques of the mechanical properties of fibers. For example, the electrokinetic properties of wool fibers, both in the native condition and with surfaces modified, have been measured to define molecular features of the surface. A typical wool fiber was found to have ionizing groups at least 50 Angstrom units apart. On the average, these groups are more strongly acidic than those of the native wool protein; this suggested that weathering of wool has resulted in oxidation of exposed cystine residues to strongly acid sulfonic acid groups.

Systematic degradation of wool, followed by chemical analysis, has provided a better understanding of wool structure and degradation mechanisms. Such results guide developmental work to impart desired wear-resistance, shrink-resistance, and other wanted properties to wool fibers and fabrics. Differences in rates of hydrolysis of wool protein were found to be caused by differences in intermolecular crosslinking through sulfur atoms. The access of wool protein molecules to chemical reagents for basic studies, or to finishing reagents for textile modification, is dependent upon the degree of swelling of wool fibers. Swelling of wool by weak acids was found to be limited by intermolecular crosslinking. The effectiveness of the WURLAN treatment is connected with chemical grafting of the WURLAN resin to protein end groups on the fiber surface. Blocking amino and hydroxyl end groups in wool prior to WURLAN treatment rendered the treatment ineffective for imparting shrink-resistance.

Isolation and characterization of wool protein depends largely upon solubilizing the proteins so they may be separated. Physical separation of wool fiber into fractions by ball milling at a low temperature, disintegration of fiber with strong hydrochloric acid at room temperature, solubilization with ethylene oxide, and partial enzymic digestion are various methods used to obtain modified protein or protein fractions that can be characterized to help us understand the properties of wool fibers.

Commercial interest exists in fabric treatments that provide oil-, water-, and stain-repellency. Excellent stain repellents exist in several types of fluorochemicals and a market is developing for garments resisting oil- and water-borne stains even though they cost appreciably more. We have been synthesizing new fluorine-containing polymers which could become important multi-purpose finishing agents for wool, emphasizing the need for compounds as effective as the available ones but costing much less. Some very promising inexpensive fluorine-containing compounds were synthesized, based on derivatives of hexafluoroacetone. Evaluation studies are now underway on several such compounds to assess their value in finishing treatments for wool. Preliminary evaluations indicated that polymers synthesized from hexafluoroacetone are durable to both laundering and dry cleaning. After several launderings treated fabrics retained excellent oil and water repellency.

In-house and domestic contract research is supplemented with a number of foreign research grants under P.L. 480. Research was initiated at the Wool

Industries Research Association in Leeds, England to determine detailed chemical structure of wool protein in relation to cystine residues. Research is centered on an isolated urea-soluble fraction obtained from peracetic acid-oxidized wool. Trypsin digestion of this fraction yielded more than 200 peptides of which about 100 occurred in substantial amounts. Fifty-six of these have been characterized by amino acid analysis and complete amino acid sequences of 27 were determined. (Most of this work was completed by the grantees prior to the grant award and forms the foundation for the grant study.) Other enzymes and also acid hydrolysis, were used to break down the urea-soluble fraction; characterization of the resulting peptides is continuing. Preliminary attempts to dissolve wool without destroying disulfide bonds were only partly successful. Trypsin and pepsin have been used to partially dissolve wool in dilute acid, in water, or in phenol.

Mild pepsin digestion of wool fibers has also been used in basic studies recently concluded at the University of Lille in France. Electrophoretic separations were used to isolate protein fragments. Protein fractions not belonging to the keratin chains but bound to keratin by disulfide bonds were found.

Investigation was initiated at the Karolinska Institutet in Stockholm, Sweden to determine essential details of the distribution of sulfur within wool keratin. X-ray absorption analysis, X-ray diffraction, and electron microscopy including autoradiographic experiments with S^{35} labeled cystine are being used. Preliminary results have demonstrated good resolution in X-ray microradiographs. They indicate that available techniques are adequate to define structural differences within the wool fiber with respect to sulfur components, and also to define the level in the fiber at which sulfur is incorporated relative to differences in structural makeup.

A research project on factors that control the migration of foreign molecules within wool fibers was concluded at the Wool Industries Research Association in Leeds, England. Wool processing requires the introduction of foreign materials into fibers, for example, dyes, acids, and bases, as well as finishing and setting agents. By studying diffusion of charged molecules they determined how mobility was influenced by size of the diffusion particle, its chemical type, and the chemical charge it carries. Migration was considerably impeded when more than one electrical charge was present on a large organic molecule such as a dye. The widely accepted explanation that particles are absorbed on the walls of pores was found incompatible with experimental data. On the other hand, evidence was found that as concentration of singly charged dye molecules increases inside the keratin structure, they tend to agglomerate and, therefore, diffuse less rapidly. If a slightly soluble alcohol is present, the agglomeration does not take place and dye mobility is greatly enhanced. This agglomeration is probably the most important cause of the accelerated dye penetration that is induced by use of fat solvents. However, solvents also produce some acceleration if the dye is in low concentration in the presence of salt. Dye molecules with more than one electrostatic charge

are not much accelerated by solvents, but they are accelerated by salts, emphasizing the importance of electrostatic interactions.

Research was initiated at the University of Allahabad in India to determine the physical properties of organic molecules and the mechanism of their interaction in liquid state. So far research activity has been confined to the development of equipment for measurement of ultrasonic absorption and calibration of the equipment.

2. Physical and Mechanical Properties. The objective measurement of mechanical properties of wool is essential to the improvement of wool products and processes. A single fiber, stress-strain curve computer has greatly increased the precision of measurement of stress-strain parameters. Subjective evaluations of wrinkling and wrinkle recovery and wool color have been materially aided by development of standards to be compared with fabric and fiber samples. Photographic standards were developed for appraisal of wrinkle behavior and good agreement was obtained among several laboratories testing the feasibility of using these standards. A series of wool color standards ranging from white toward yellow in five equal steps, and another set of five standards of similar degree of yellow but with variable grayness, were assembled and tested cooperatively through Committee D-13 of the American Society for Testing Materials. A manufacturer is being sought to make a number of comparators to be used in industrial trials preliminary to writing specifications for an ASTM test method. Also in cooperation with Committee D-13 ASTM, a method for testing the strength of wool using cotton-type bundle testers was developed and is under consideration as an ASTM standard method. A more precise mathematical description of the deformation of fiber low-strain torsional and tensile elastic properties after swelling in formic acid solutions was developed. Crease height measuring equipment was developed and demonstrated to be highly accurate and related to crease quality (i.e., the sharpness of crease).

3. Effects of Radiation and Other Physical Forces on Wool. The destructive effects of heat, light, oxygen, and water vapor on wool and wool products are under continuing investigation. Irradiation of wool induces rupture of disulfide bonds through a free radical mechanism as evidenced by electron paramagnetic resonance and the appearance of a slight green color. Gentle heating causes recombination of ruptured disulfide bonds and the disappearance of the green color. The cystine radical formed by rupture of the disulfide bond can also be made to disappear in the presence of oxygen, but the process is slow and reversible. Water vapor causes a rapid, irreversible disappearance of both cystine and tyrosine radicals.

The effect of sunlight on wet wool is twofold; both bleaching and yellowing occur and may be unrelated. A series of short- and long-term experiments were conducted in which the same wool fabric was exposed to sunlight in Melbourne, Australia and Berkeley, California. The fabric yellowed in Melbourne but was bleached in Berkeley, from which we may infer variability of sunlight or atmospheric differences.

A basic study related to the observation that free radicals are produced in wool by irradiation was initiated using methylene blue as a model molecule and following the effects of irradiation by use of electron paramagnetic resonance and optical spectroscopy. Irradiation-induced free radical formation and subsequent decomposition of methylene blue were investigated. In alkaline solutions, methylene blue progressively loses methyl groups and its demethylated products are also reduced to form free radicals. In wool the initial effects of irradiation are to produce many different free radical species. Studies will continue in elucidating the various effects of irradiation on wool.

B. New and Improved Products and Processing Technology

1. WURLAN. WURLAN is the name given to the Department-developed interfacial polymerization application of polyamides to the surface of wool fiber and fabrics to make them shrink-resistant and machine washable. WURLAN-treated fabric is currently being produced at an annual rate in excess of one million yards of material. The successful commercialization of WURLAN-treated fabric was followed by an even more rapid commercial adoption of WURLAN treatment of wool top. Treated top is commercially spun into yarns that are available in large quantities for commercial production of knit goods and for retail distribution of knitting yarns.

Research is continuing on problems related to cost of treatment, the setting up of trade-wide wash standards, and development of more compact and efficient WURLAN-treating equipment. Appraisal of alternative reagents and conditions of application continues. Informal cooperative work with industry resulted in several cost-reducing processing improvements and extended production runs were made with a new reagent (hexanediol bischloroformate) which, according to laboratory tests, should eliminate a mechanical processing problem associated with interfiber bonding and resin dusting from the finished product. A redesigned top roll for the diamine padding step has increased extraction efficiency reducing the solution pickup by top which also reduces problems of fiber bonding and resin dusting.

2. Stretch Woolens. Laboratory tests indicate that stretch fabrics woven from 100% wool yarn are feasible. Stretch yarns were prepared by WURLAN setting of a coiled configuration obtained by application of twist-on-twist to a plied yarn, treating the yarn and then back twisting past the zero point to a standard twist. Good elastic recoveries were obtained under conditions that demonstrated the stability of stretch to wet processing.

3. Yarn and Fabric Construction. Modern high-speed processing of wool fiber into yarn requires lubrication. Proper fiber lubrication must be developed for chemically modified as well as untreated wool and mohair. Resin dusting of WURLAN-treated top has been bothersome and a series of commercially available fiber lubricants representing a wide range in properties were studied to determine their effectiveness in reducing fiber-to-fiber and fiber-to-metal friction in pin drafting. Overtreated wool was used to give excessive resin

dusting and lubricants were evaluated for their reduction of friction, anti-static action, softening effect, solubility, and scourability. Two lubricants were found that virtually eliminated the dusting problem. However, other properties did not appear as good as they might be and the study of lubricants will continue.

Research conducted under a P.L. 480 grant on the mechanism of lubrication of worsted yarns was concluded by the Hosiery and Allied Trades Research Association in Nottingham, England. Uniformity of stitch length in knitted fabrics is affected by yarn-to-yarn friction and yarn-to-metal friction, which in turn may be modified by lubrication. Different waxes were evaluated as to lubricating efficiency and wax pickup. Pure paraffin wax with 125-130° F. melting point, applied from solid form, was the most satisfactory lubricant studied for worsted yarns, whether they were dyed or not. When paraffin wax was applied correctly, no advantages were obtained by use of modified waxes containing silicones. Particles of wax were picked up by the yarn and partially transferred to surfaces of succeeding guides and redistributed on the yarn. It was postulated that a thin layer of wax melted at the sliding yarn-wax interface by frictional heating with a layer of wax remaining unmelted on the guide or pillar. Friction was independent of yarn speed and also temperature up to the point where the wax melted. Above the melting temperature, solid wax on the guide surfaces was lost and friction increased even beyond that of unwaxed yarn and knitting consequently was impaired.

Fabrics treated chemically to improve setting showed a higher crease recovery if the creasing and chemical setting were both accomplished with the fabric at 20% moisture. An experimental interaction between chemical setting and fabric construction was observed in contract research underway at the Harris Research Laboratories in Washington, D. C. In these studies, chemically-set tropical worsted slacks wrinkled more in service than unset fabric but worsted flannel did not behave in the same way. The chemically-set flannel slacks retained creases better than unset and were also less baggy. In these studies so far, 66 experimental fabrics have been finished and their various construction parameters determined. These fabrics differ in fiber content, yarn construction, fabric construction, and chemical treatment, and are being evaluated for wear wrinkling. Laboratory tests and subjective appraisal after trial wear of the garments are being made of a selected portion of the fabrics. Crease recovery tests were made on all the fabrics. The shear properties of all fabrics were determined for both warp and fill directions. Construction factors and yarn parameters affected shear properties of fabrics. Distortion angles for WURLAN-treated fabrics were found lower than for the untreated or flat set fabrics, which indicates less yarn mobility in the WURLAN-treated fabrics. About one-fifth of the fabrics that were constructed were made into garments and are appraised for wrinkling after various periods of wear. Laboratory tests at 90° F. are being made to appraise service of these fabrics under severe conditions. Wrinkle recovery properties were better with woolen fabrics than worsted, and heavier fabrics were better than lighter ones.

4. Fiber and Fabric Treatments to Make Care Easier. Promising leads for a new method of application of multiple-purpose finishing agents were obtained on a laboratory scale. It is based upon the crosslinking of preformed polymers on wool. This technique is being used with a number of highly reactive resins. For example, modified polyethylene resins impart a highly effective degree of shrink resistance to wool fabrics. The use of water emulsions would minimize any potential problem of stream pollution which might otherwise result from large-scale solvent-base treatments. The polyethylene resins currently used in laboratory studies are more expensive than the WURLAN chemicals but would probably be less expensive if other large-scale industrial use developed. Excellent shrinkage protection was obtained with no impairment in desired textile properties. Further pilot plant developmental studies are required before this process can be commercialized.

Contract research on chemical modification of wool to increase drying rate was concluded at the Research Triangle Institute, Durham, North Carolina. Substantial grafting of vinyl monomers to wool was achieved by direct radiation of wool with gamma rays in the presence of the monomer and by irradiation of the wool followed by addition of monomer. Styrene, acrylonitrile, and stearyl methacrylate were successfully grafted. Swelling of wool was necessary for radiation-induced grafting. Wool-water relationships that were investigated in grafted and normal wools included drained water content and equilibrium sorption properties. In general, only modest decreases in equilibrium moisture contents of polymer-grafted wools were obtained. However, there was a great effect on the drained water content that indicated the surface of the treated wool was more hydrophobic than untreated wool so that surface water would run off much more rapidly than from untreated wool. The treatment also reduced the rate at which water diffused into wool fibers.

In contract studies conducted by the Harris Research Laboratories in Washington, D.C. methods were developed for measuring luster in wool fibers. The equipment allows for measurement with a single wool fiber or sets of fibers measured several at a time. Preliminary examination of commercial fabrics showed that luster depends both on previous mechanical processing and depth of shade of dye. Woolen outerwear fabrics which derive luster from oriented pile fibers were examined, treated and tested for improvement and durability of luster. Application of polymer finishes by the WURLAN treatment produced a small improvement in luster. Larger effects on luster, both before and after steaming, were obtained by reducing and setting treatments using monoethanolamine. Brushing fabrics while moist with the treating solution orients the pile and further improves the luster effect. Although intrinsic luster of fibers may be increased by chemical modification, it appears more likely that greatest improvement in luster will come through improved setting of fibers properly oriented in the fabric structure.

Interrelationships of different wool finishing processes are investigated under a P.L. 480 research grant to the Textile Research Association in

Helsinki, Finland. Research has been conducted on both top dyed fabrics and fabrics that are piece dyed. These investigations include plain weaves, Panama weaves, and twill weaves made from coarse, medium, and fine wool. Factors influencing the evenness of dyeing and the setting of fabric to a smooth surface were studied. Fabrics with quite different properties were produced, depending upon whether or not the wool fibers in the fabric had been present during top dyeing. Top dyed fabrics with the fibers present were superior to others in most cases. It appears possible that pretreating of wool fibers which are to be subsequently dyed in fabric form would minimize variations in width, shrinkage, and color shade. These properties are more variable in fabrics that are piece dyed than those dyed at the top stage.

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AREA NO. 5. CITRUS AND SUBTROPICAL FRUITS--PROCESSING AND PRODUCTS--WESTERN LABORATORY

Problem. The economic stability of the citrus and subtropical fruit industries in the Western Region is dependent upon effective utilization of fruit that cannot be accommodated on the fresh fruit market. The utilization of surplus or wholesome but blemished fruit provides the margin necessary to assure adequate returns to the farmer and continued development of stable markets. Ineffective utilization of products and continuously increasing processing costs are resulting in decreased returns to the growers. The California-Arizona grapefruit industry is encountering difficulty in disposing of both fresh fruit and processed grapefruit products. The pineapple and subtropical fruit industry in Hawaii must find practical methods for processing its products for export in order to prevent the accumulation of burdensome surpluses. The navel orange industry in California is hampered by the unavailability of satisfactory processes for the utilization of navel oranges. Juice extracted from early fruit, and during some seasons from almost all of the navel oranges, contains substances that impart an intolerable bitter flavor to juice products after mild heat-processing or after standing at ambient temperature for a short time. Large new plantings of navel oranges may be expected to aggravate the utilization problem. Deterioration of the flavor and color of these and other processed citrus and subtropical fruit products imposes severe limitations upon the economic stability of the industry.

Information is needed on the chemical composition of citrus and subtropical fruits and their products and byproducts as a basis for the development or application of new and improved methods of processing; and for the production of new and improved food and industrial products and pharmaceuticals. Special attention needs to be given to the nature of the chemical changes involved during pre-treatment, processing and handling which lead to the formation of off-flavors, -colors, and -odors in processed products.

USDA AND COOPERATIVE PROGRAM

In the Western Utilization Research and Development Division, a concentrated program of fundamental research on citrus and subtropical fruit and its application to industry problems is conducted at the Division headquarters at Albany, California; at the Fruit and Vegetable Chemistry Laboratory in Pasadena, California; at the University of Hawaii, Honolulu; by contracts at Pasadena, California and Tucson, Arizona; and, under a P.L. 480 grant, in Bogota, Colombia. Investigations are conducted on the composition of citrus essential oils, flavonoid compounds and other citrus constituents that are related to off-flavors and darkening of citrus products, the natural flavor components of oranges, enzyme systems that are involved in the appearance and disappearance of constituents and structures of plant tissues,

constituents of dates that affect the quality and stability of products, and the application of findings of such research to the development of new and improved citrus, tropical, and subtropical fruit products.

The Federal program of research in this area totals 19.4 professional man-years, including contract research equivalent to about 1.6 professional man-years per year and two scientists whose salary is provided under Memorandum of Understanding by the Lemon Products Technical Committee. Of the total, 12.2 are assigned to investigations on chemical composition and physical properties and 7.2 on new and improved food products and processing technology. In addition, the Division supervises two research projects supported by P.L. 480 grants.

PROGRAM OF STATE EXPERIMENT STATIONS

State stations engage in both basic and applied research on the utilization of citrus and subtropical fruits with the objective of expanding markets and increasing utilization of these crops. Citrus, oranges, grapefruit and tangerines are held in special environmental control chambers and the various combinations of temperature, humidity and airflow are studied to determine subsequent effects on quality. Efforts to reduce decay during storage and transit lead to research on the basic physical, biochemical and physiological changes which occur during handling and marketing. Factors influencing quality of mature avocado fruits are evaluated by study of the respiratory rate, various physiological disturbances, ripening rate and external and internal quality of fruits. Non-destructive physical measurements of quality are sought.

Product research and development includes study of processes for canning grapefruit sections. Special attention is given to problems of texture, structure, flavor and color. The characteristics of commercial frozen Florida orange concentrate and superconcentrate are determined at intervals and used to establish characteristics of these products. Characteristics of canned and frozen concentrated juices are studied initially and after storage at elevated temperatures. Efforts to extract, separate, identify, and determine quantitatively each of the volatile components responsible for the natural flavors and occasional off-flavors in citrus fruits, citrus oils and processed citrus products continue. The determination of the relationships of all components to the total flavor and aroma presents numerous unsolved problems.

Certain properties of avocado polygalacturonase and papaya pectinesterase are being studied. In addition, the enzymes of the fig latex are being isolated and characterized as to molecular weight, activity and amino acid composition.

Work with subtropical crops such as guava, mango, soursop, banana, pineapple, coffee and plantain involves development of processes for preservation of the delicate and characteristic flavor of these fruits. Production of freeze-dried

products of high quality and good storage life is under study. Products such as banana puree, fried snack items, fruit powders, candied items, and nectars are receiving attention.

The Hawaii and Puerto Rico stations conduct research to strengthen their coffee industries. These studies include research on the microbiology of the coffee fermentation process, on drying coffee and on the quality and acceptability of the final product. Basic equilibrium moisture content data for parchment and green coffee are being developed to guide design of an experimental system which uses solar energy for drying coffee.

Research designed to recover or make useful products from citrus and pineapple wastes is in progress. Other work is directed to conversion of citrus terpenes to useful chemicals and to use of isolated cultures from natural sources to produce glycerol and glycols from citrus wastes by fermentation. Feasibility studies on the preparation of livestock feeds from farm product and distillery, cannery and brewery wastes continue with materials selected for study being pineapple, citrus and pigeon pea cannery wastes, spent grains and blackstrap molasses.

The research effort devoted to citrus and subtropical fruit utilization research is about 19.6 professional man years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Citrus Composition. Citrus components are being isolated and identified to provide foundation knowledge that will help improve citrus products and processes. Over the past several years, the major polyphenolic compounds of citrus fruits have been isolated and identified, and a number of previously unreported compounds have been discovered. Naringin had been thought to be the only important polyphenol of grapefruit. Now we have isolated and identified six other major polyphenols from the edible portion (endocarp) of grapefruit by enzymic hydrolysis. These findings are of major significance not only to citrus processors but also to geneticists, botanists, and plant physiologists, since they shed light on the distribution patterns in various citrus varieties.

The chemical structure of nootkatone, a sesquiterpene ketone discovered in grapefruit and other citrus oils last year, has been elucidated. The structure satisfied all the chemical spectral evidence developed, including elemental composition and IR, UV, and NMR spectra. Other oxygenated sesquiterpenes appear in lemon oil but in lesser amounts.

Research on lemon oil and lemon juice is supported in part by the Lemon Products Technical Committee. Data on lemon juice composition collected over five seasons were subjected to multiple regression analysis; the equations obtained are useful for predicting the expected levels of citric

acid in unaltered lemon juice. Sterol, carotenoid, and citric acid values in 26 samples of commercial lemon concentrate were determined. The average values of these components in fresh coastal and fresh desert lemons were not significantly different from the average values of their concentrates.

Preliminary results indicate that acidity decreases in lemons during periods of low humidity (such as hot dry winds), but it increases to its previous value when the humidity is increased. A 5% increase in citric acid content of lemons was obtained by increasing the humidity around a tree with an overhead water spray. This new information, along with the multiple correlation of analytical data, should lead to a more precise definition of authentic lemon juice in terms of its citric acid content.

2. Bitter Constituents of Citrus. Extensive work has been conducted on the flavonoids and limonoids, two types of compounds that are natural constituents of oranges and grapefruit and that can cause bitterness in these fruits. Desoxylimonin, known previously only as a synthetic derivative of limonin (a bitter principle of navel oranges) was isolated from grapefruit seeds and identified. It was found to be tasteless. If a mechanism could be found that would convert limonin to desoxylimonin, it would suppress bitterness in navel orange products.

In addition to limonin, nomilin and obacunone are compounds known to cause bitterness in navel orange juice. Citrus processors need a simple, practical assay method for these three compounds so that lots of fruit high in bitterness can be diverted to nonfood uses. Contract research has been initiated at Stanford Research Institute, Menlo Park, California, to develop such analytical methods.

3. Fruit Flavor Components. We have greatly improved the resolution, capacity, and sensitivity of gas chromatographs in order to facilitate the study of hydrocarbons and oxygenated compounds found in citrus and deciduous fruit volatiles. The volatiles from single pieces of fruit can now be analyzed. Molecular weight and structural information can be obtained with microgram and even smaller quantities by direct coupling of gas chromatography columns to a fast-scan mass spectrometer.

Chemical studies emphasize methods for the recovery of volatile components of fruit juices. In volatiles from orange juice prepared so as to minimize or eliminate contributions of peel oil, 35 compounds have been identified. Ten of them are newly identified from this source; 7 others had been only tentatively identified in orange juice prior to this work. Aroma contribution of individual compounds is being evaluated by test panels.

Research on stabilization of flavor concentrates of tropical fruits is supported by a P.L. 480 grant to the Institute of Technological Investigations in Bogota, Colombia. Aromatic components have been solvent-extracted from the guava, fractionated by distillation, and analyzed by gas chromatography. A novel method of flavor extraction is being studied: Fruit pulp is layered

with powdered sugar, which partially dries the pulp, and in the process the sugar is converted to syrup which contains appreciable amounts of volatile flavoring substance. Essence is then stripped from the syrup by distillation and concentrated. An essence concentrate that preserved the pleasant character of the fresh fruit was prepared from curuba pulp in this way.

4. Composition of Dates. Three polyphenolic acids related to enzymatic browning reactions in dates were isolated and their structures determined. Other organic acids are being identified. Of ether-soluble acids in immature dates, about 75% was malic acid. Phosphoric, citric, and galacturonic acids were present in uncombined form. Shikimic, glycolic, and galacturonic acids were in bound form and represented about 4%. The presence of bound shikimic acid and the absence of quinic acid suggest the absence, also, of quinic acid dehydrogenase enzyme activity. The unique presence of caffeoylshikimic acid suggests that a metabolic activity may be present in dates that is different from that in most common fruits, which contain significant amounts of quinic acid. Four flavans that were enzymic browning substrates were found in immature dates but not in mature dates. The disappearance of such compounds during maturation suggests that enzyme-catalyzed browning is partly responsible for the characteristic golden brown color of ripe dates.

5. Pharmacological Investigations of Citrus Products. The metabolism of caffeic acid depends upon micro-organisms in the gut rather than upon endogenous reactions within the animal body. This observation led us to initiate studies of metabolism *in vitro* that may lead to a more basic understanding of the metabolic fate in animals of minor phenolic constituents of citrus. Freshly voided animal feces were incubated with various substrates, such as the flavonoids and phenolic acids common in citrus fruits and other plant materials. Preliminary indications of the nature of the primary metabolic breakdown of quercetin were obtained. Identification of several biofermentative phenolics produced from flavonoid nuclei is in progress. Other common phenolic compounds such as dihydroxyphenylalanine, xanthurenic acid, D-catechin, phloroglucinol, and rhamnetin are now being investigated by this procedure.

B. New and Improved Food Products and Processing Technology

1. Citrus Products. Low-calorie sweeteners can be made by converting the flavonoids naringin and neohesperidin to their dihydrochalcones. Improved methods have been developed for the direct conversion of commercially available and relatively cheap naringin to the much more expensive neohesperidin and its dihydrochalcone. This direct conversion improves the outlook for the practical production of a food sweetener. Requests for samples and information on their preparation indicate a continuing commercial interest in this development.

Compositional studies of the carotenoid pigments of citrus fruits have revealed the presence of a red-orange pigment, citranaxanthin, which is the first known naturally-occurring carotenoid with a methyl ketone side chain.

Citranaxanthin has provitamin A activity and good stability in vegetable oils, oil suspensions, and emulsions. It may have use as a food color.

Contract research was initiated at the University of Arizona to develop new food products from desert grapefruit, with particular emphasis on blends of grapefruit and other fruit juices, new diced and segmented grapefruit products, and grapefruit juice or puree for use as an ingredient in other food products. Preference levels for sweetness, sourness, bitterness, and saltiness were evaluated, and an acceptable standard as compared with juice was developed (0.02% naringin, 0.1% acid as citric, and 13° Brix). A blend of 25% seedless grape juice and 75% grapefruit juice was prepared and filtered to give a clear product. It had excellent taste and eye appeal. Tests with firm, early-season peeled grapefruit indicated the possibility of adapting commercial equipment to the mechanical dicing of grapefruit. Microwave heat pasteurization of diced grapefruit in plastic pouches showed promise as a means of extending the shelf life of refrigerated grapefruit salad. Enzymic debittering of grapefruit juice with cellulase increased soluble solids content and gave higher yields of product with lower bitterness, better cloud, and fewer particles large enough to settle during storage of the product.

2. Tropical Fruit Products. In Hawaii, interest is growing in diversification of fruit products. Research on the technology of fruit processing and demonstrations of good practices at our field station in Honolulu are aiding this industry. Dehydrated banana products of satisfactory quality were prepared by air drying, freeze drying and drum drying, and the stability of the dried products was tested at three different storage temperatures. Two varieties of bananas have been tested for processing quality; the use of sulfur dioxide to prevent deterioration was included in the experiments. Juice and puree concentrates for remanufacture into jellies and preserves were prepared from guava, papaya, and passion fruit. Pectin-degrading enzymes had to be used to reduce the thickness of guava and papaya purees so they could be concentrated. Passion fruit puree did not require this pretreatment. Starch-splitting enzymes were used to control the consistency of poi, which tends to thicken when held for a few days in markets. These preliminary experiments indicate the possibility of fairly precise control of the consistency of poi, which has a sizable market in Hawaii. The use of poi in baby food, particularly to avoid grain-flour allergies, may widen the market for this product.

3. Foam-mat Drying. In cooperation with the Southern Utilization Research Division at Winter Haven, Florida we conduct research on foam-mat drying of orange and other citrus products, and informal cooperation continues with industry on the commercial application of foam-mat drying, particularly in connection with the development of equipment. In-house activity during the past year has consisted largely of studying factors such as solids content, foam density, stabilizer level, foam temperature, and dryer temperature for drying various liquid foods. Berry purees foam-mat dried with sugar added retain excellent flavor and aroma. A non-caking foam-mat-dried edible molasses was prepared by bin drying in trays to 1% moisture content a product that

had been foam-mat dried to about 2-1/2% moisture. Cranberry juice foam-mat dried with sugar added reconstitutes readily to a product with quality similar to that of cranberry juice available in domestic markets.

It has now become possible to foam-mat dry citrus to less than 1% moisture by using fine-bodied foams. This allows us to omit the expensive, awkward, and bulky in-package desiccant bag previously needed to avoid caking. Another improvement relates to increasing the powder density. The powder can be converted to small flakes by squeezing it between warm steel rolls. In this way the bulk density may be raised from 0.3 to 0.8 gm. per ml. This now allows a manufacturer to put 70% more juice solids in a package by foam-mat drying than frozen juice concentrate.

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AREA NO. 6. DECIDUOUS FRUIT AND TREE NUTS--PROCESSING AND PRODUCTS--WESTERN LABORATORY

Problem. Fruits and nuts are valued for their unique flavor, color, and natural vitamin content. In the period of abundance at harvest time, markets are glutted and growers often do not get an adequate return. Crops are perishable, and processing to preserve their unique qualities is difficult. No processed fruit retains completely the fresh values, although many highly acceptable products exist and about half of the fruits and nuts marketed in the United States are processed. Processing makes these commodities available to consumers the year around, and has opened new markets for producers. The proportion of processed commodities is steadily increasing but is dependent upon a continuing flow of new knowledge. Processing to preserve color, flavor, and texture presents many problems generally, and each new product requires the application of much scientific and technological skill.

The freezing process for preserving certain fruits keeps the products excellent at near fresh fruit condition. In spite of the gains in quality realized in freezing, many unsolved problems remain. The enzymatic browning of frozen peaches and the sloppy texture of frozen strawberries on thawing are two good examples.

Frozen fruits require expensive low-temperature storage and transportation facilities. The expense is greatly reduced by removing a portion of the water from the products. Orange and other fruit juice concentrates are well established in U.S. markets, and dehydrofrozen apple slices (rapid drying to 50% bulk weight and then freezing) are just becoming well established. Many other fruits and fruit juices should be amenable to concentration. Products of this type, however, are not so well adapted for export as those which do not require refrigeration.

The maximum weight reduction can be achieved through dehydration. The drying of fruit juices has been successfully accomplished by the vacuum puff drying and foam-mat drying processes. The latter is under intensive study, because it can be carried out at atmospheric pressure and consequently offers economy in processing. This process must be worked out in detail for many, as yet untried, fruit purees and juices and on pilot-plant scale for those products that show promise. Flavor recovery and the incorporation of recovered flavor in solid carriers for addition to the dried products require technological and basic chemical study. Essence recovery techniques developed for fruit juice concentrates are not completely satisfactory for this purpose.

Dried and canned fruits are now widely used in the U.S. and abroad. The popularity of dried fruits overseas and in this country would grow if stable,

higher moisture dried fruits were available and if lower levels of sulfur dioxide could be used without loss of quality.

Container costs for canned fruits limit the shipment of these products overseas. A solution of the container problem may be found in the use of lightweight fiber, foil, or plastic containers and aseptic filling procedures.

Fruit growers need new varieties of tree fruits and berries suited to processing and resistant to diseases endemic to each region of production. Utilization research is required in cooperation with farm research to assure growers of a market for fruit in the processing industry.

USDA AND COOPERATIVE PROGRAM

In the Western Utilization Research and Development Division, a broad program of basic and applied research on deciduous fruits and tree nuts is conducted at the Division headquarters at Albany, California; in field stations at Pasadena, California and Puyallup, Washington; by contracts in Davis and Los Angeles, California, Fort Collins, Colorado, and Geneva, New York; by grant at Cambridge, Massachusetts; and by grant funds under P.L. 480 in Israel and India. Fundamental research is conducted on fruit constituents that are involved in the flavor, color, and texture of fruit products, and includes development of laboratory tools to isolate and characterize individual components, investigation of such components as they occur naturally and as they are altered by operations involved in preservation, and the relationships between the components and the product values being preserved. Applied research is conducted to develop new and improved processes and products that will increase utilization of fruits and tree nuts, including the development of high-quality concentrated and dehydrated products and more stable shelled tree nuts and the selection of improved processing varieties. Pioneering research on plant enzymes is also conducted.

The Federal program of research in this area totals 41.3 professional man-years, including two scientists whose salaries are provided by two cooperators (Dried Fruit Research Advisory Committee, whose membership represents the California Raisin Advisory Board, the Dried Fig Advisory Board, the California Prune Advisory Board, and the Dried Fruit Association of California; and the Walnut Control Board - one each), under Memoranda of Understanding; six grants and contracts providing research at a rate of approximately 4.7 professional man-years per year. Of this number, 25.8 are assigned to investigations on chemical composition and physical properties; and 15.5 on new and improved food products and processing technology. In addition, the Division sponsors basic research on fruit by means of three P.L. 480 grants.

PROGRAM OF STATE EXPERIMENT STATIONS

The States have a continuing and effective program of research on fruit processing and fruit products. Fruits possess unique flavor, color and nutritive qualities which make it desirable although difficult to preserve their unique qualities.

Fruit utilization research begins with evaluation of varieties and selections for processing quality as a service for breeding programs. In addition the relationship of other production and cultural practices to the quality and utilization of the finished products are determined. The biochemical changes associated with post-harvest storage and ripening are studied in an attempt to elucidate the metabolic reactions associated with ripening and to devise means of their control. Respiratory activity of fresh fruits is determined and used as an indication of package environment and to guide package selection. Increased use of mechanical harvesting equipment has been found to directly affect the quality of the fresh fruit and processed products.

Increased interest in pesticide residues on food products has led to the initiation of three regional projects. This regional research is centered around development and evaluation of procedures for reduction or elimination of residues in processed foods. Fruits and fruit products are among the commodities being studied.

The chemical composition and physical properties of certain fruits are being investigated in detail. The color and pigments of fruits are of special interest. Basic research on identification of the polyphenols of fruits and their role in enzymatic browning is continuing. The ultimate texture and reconstitution properties of fruit products are related to the properties of the pectic substances contained in the fruit initially. For example, the polysaccharides of the cell wall and other tissues are under investigation because these compounds are so important to texture of the products. Ethylene metabolism and its role in fruit ripening is under study.

The chemistry of flavor continues to advance with improvements in gas chromatographic procedures. Compounds which have the characteristic properties of fruit flavors are isolated and identified by this technique. Considerable effort is devoted to determining their significance in the flavor response to specific fruit flavors. These findings are correlated with taste evaluations and, through this process, some insight into the development of undesirable flavor is gained.

As previously indicated, study of enzyme mechanisms and properties constitute an important and continuing phase of basic research on fruits. More applied phases of investigation deal with such problems as development of off-flavors in frozen fruit products, enzymatic browning of fresh tissue and methods for control of this form of browning.

Investigations of the ecology, taxonomy and physiology of yeast, molds, and bacteria involved in food fermentations and spoilage are designed to help

understand how microbes occur in nature, how they get into foods, and how they bring about either desirable or undesirable changes. This information is used in control of fruit spoilage and in developing and controlling desired fermentation processes such as those involved in wine manufacture. For example, the role of specific micro-organisms believed to cause softening of brined cherries is under continuing study. Comprehensive studies deal with the fermentative conversion of fruit juices to wine. These studies range from fruit composition factors through study of yeast growth factors important in the fermentation to changes in wine during aging. The highly specialized study of the microbiology of olive fermentation continues. Other applied investigations are concerned with evaluation and enumeration of bacteria found in frozen fruit products.

Research directed to development of new or improved fruit products and processing technology continues to be a major part of the fruit utilization program. This work extends from study of the processing quality and suitability of selections and varieties to characterization of the chemical, physical and storage properties of a new fruit product. Basic studies deal with thermal processing requirements and include relationships to mechanism of heat transfer and thermal breakdown of various fruit constituents. Process design including methods, equipment, and plant layout receive study. Newer methods of freezing, dehydrofreezing, freeze-drying, irradiation and dehydration of fruits are under continuing investigation. Effects of chemicals, hydrocooling, refrigerated storage and controlled atmosphere storage and holding are evaluated in terms of the changes in product texture or structure, color and flavor. Product research is designed to provide basic information on product potential and functional properties. Development of processes or products to improve the utilization of fruits involves work on: dehydrated fruits; apple sauce; frozen fruit pies; apple-fruit juice blends; sherry wines; brined cherries; low sugar apple jelly; macadamia nuts; peach concentrates; and grape products. Research on fresh and roasted macadamia nuts elucidated the quality characteristics of these products and defined optimum conditions for storage.

The total research effort on fruit utilization is approximately 59.1 professional man years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Fruit Pigments. Red, purple, and blue fruit colors come from phenolic compounds known as anthocyanins. Fruits lose color when anthocyanins change to colorless leucoanthocyanin compounds. From model systems of synthesized flavylium salts and phloroglucinol, resorcinol, and catechin we postulated that the reaction is a condensation of the pigment with other natural phenols. Further studies with natural catechin strengthened the hypothesis. Theoretically ascorbic acid and simple sugars, such as fructose, could destroy color by similar reaction. Studies at the University of Delhi in India, supported

by Public Law 480 funds, have shown that anthocyanidins react with ketones and lose color in fruit products. Leucoanthocyanidins were isolated for study from pears, prunes, quince, peaches, cherries, and persimmons in this work.

Contract research at UCLA established that the principal polyphenolic proanthocyanidin of avocado is a condensation product of two phenolic compounds. The proanthocyanin isolated from avocado is a new type of natural flavonoid compound. Its structure led them to expect that red anthocyanin pigments would develop under acid conditions and this was demonstrated.

Phenolic components of apple juice are involved in a protein complex that eventually settles out of clarified juice. Chemical examination of washed sediment revealed a phenolic component. Although it contained little nitrogen, acid hydrolysis split off a number of amino acids indicating protein was involved. Catechins and leucoanthocyanidins were observed in the original clarified apple juice but could not be detected after sedimentation. These observations were obtained in contract research at Colorado State University.

The degradation of carotenoid pigments (yellow, orange, and red) was studied in acetone or methanol solutions with hydrochloric acid. Carotenoids vary widely in reactivity with acid. Reaction products of the degradation were identified. Color loss of both anthocyanin and carotenoid pigments in fruit products has generally been considered to be oxidative, although acids have been implicated in carotene transformations. Other mechanisms for anthocyanin loss were suggested by other investigators in this field but with no clear-cut understanding of the type of chemical reactive groups involved and no knowledge of the nature of the products of the reaction. Information gained from in-house, contract, and grant research is contributing a deep chemical understanding of condensations involving anthocyanins and other natural compounds. This new knowledge should ultimately lead to practical controls for the color of processed fruit products.

2. Enzymic Browning of Fruit. In the enzymic browning of fruit, phenolic constituents are converted to colored compounds. Adding a methyl group to such compounds by the enzyme methyltransferase system blocks enzymic browning reactions. Basic investigations are in progress on the methyltransferase enzymes and on the phenolic substrates of the browning reaction. Molecular conformations of quinic acid, four structural isomers of chlorogenic acid, three of isochlorogenic acid, and five of dehydroquinic acid were determined by nuclear magnetic resonance spectroscopy and optical rotatory dispersion studies. Catechol-O-methyltransferase from plant sources was fractionated and purified so that its specific enzymic action could be more narrowly characterized. Differences in the specific activity of plant O-methyltransferase from animal O-methyltransferase were determined. Differences in the molecular positioning of methyl in phenols by the enzymes serve a useful function in organic synthesis of methoxy phenolic compounds and may ultimately make possible better control of fruit browning.

These basic findings were immediately applied to preservation of peeled apples for bakery use. Dipping peeled apples in an acid solution alters the surface pH and allows native methyltransferase enzymes to inhibit browning so that the product may be held under refrigeration for several weeks without browning. An earlier commercial process prevented browning by treatment with sulfur dioxide in the dipping solution. By use of the pH adjustment related to methyltransferase activity, it is possible to materially reduce the sulfur dioxide concentration so that flavor and texture of the treated apples were substantially improved.

Studies on enzymic browning of fruits are also conducted at the Hebrew University in Jerusalem, Israel under P.L. 480 grant funds. In these studies the enzymes that change colorless phenolic compounds to reddish brown pigments are being isolated from various fruits. These enzymes, phenolases, are contained in the chloroplasts and mitochondrial structures of plant cells and are released as the fruit ripens. Their release accounts for the increasing tendency of fruit to brown as it ripens. Phenolases were isolated from apples, peaches, and for comparison from lettuce seeds, sugar beet leaves, and potato tubers and characterized with regard to their browning activity with various phenolic substrates and the dependence of their activity on pH. Improved procedures were developed to remove the enzymes from particles in the plant cell to which they are tightly bound. Three different enzyme fractions were extracted from chloroplasts of apple. Chemical inhibitors of phenolase were found and are being used to obtain more detailed knowledge of the mechanism of inhibition. These investigations were extended to nine different varieties of apples and four of peaches. The enzyme systems differed in different varieties of apples, but were the same in all varieties of peaches.

3. Chemical Origin of Plant Structural Tissue. Investigations have been made on the cell wall and other structural tissues of plants; how they form and degrade during growth and ripening of fruits. Myoinositol is an active depot of carbohydrate material. Because it changes to pectin, hemicelluloses, and cellulose, it must be important in the development of texture in ripening fruit. In-house research on myoinositol was concluded while basic investigations in this field are continuing by grant research. A grant was made to the Cell Biology Laboratory, Harvard University to obtain information on the formation and physical structure of fruit tissue cell walls. They are working on the internal morphology of microtubules of plant cells. Electron microscopy provided evidence supporting the contention that these minute structures are tubular and are composed of 13 subunits linked in a circular arrangement to form a central bore or channel. Protoplasmic streaming in cells is also under observation. This streaming appears to result from a contractile system that does not involve the microtubules. The liquid phase of the main streaming channel is connected to the surrounding environment by a complicated interlinking of tributaries with only the slime layer as an external barrier. Investigations of the streaming phenomena will be concluded because they did not reveal a connection with the microtubules.

A P.L. 480 research grant has been made to the National Taiwan University, Taipeh, Taiwan, China for basic investigations on the formation of hemicelluloses and nitrogenous compounds associated with cell walls and of intermediates and enzyme systems involved in hemicellulose synthesis.

4. Fruit Flavor Components. Fractions of the volatiles from apple essence were separated with a packed column and a non-destructive detector. The fractions were evaluated for aroma and their structure investigated. Characteristic apple aroma seems to come from only a few individual compounds whose structure and sensory characteristics are being established. Relative odor intensities of apple essence fractions were determined by a combination of gas liquid chromatography separations and test panel threshold measurements. Panelists sniffed each fraction as it was separated by liquid-gas chromatography. The fraction with the most intense odor (i.e., the one detectible at the most dilute concentration), was the fraction present in least amount. Two samples of Delicious apples, one each of Jonathan and McIntosh apples, and a mixture of juice, including those four samples plus juice from Rome Beauty and Northern Spy, were compared by this separation and evaluation technique. For all five of the samples tested, the judges picked the same small chromatographic fraction as being the best apple aroma. Chromatographic estimate of concentration of the intense fraction was determined for apple essence stored under different temperatures. The results indicate the possibility of using the concentration of this fraction as a basis for establishing processing control limits for desirable aroma in apple products and illustrates the possibility of making objective instrumental measurements that have sensory meaning.

5. Pioneering Research. A Pioneering Research Laboratory conducts basic research within the Western Utilization Research and Development Division to discover, identify, and characterize the enzyme substrate systems responsible for formation and disappearance of plant constituents and structures.

Ethylene Metabolism in Plants. When ethylene is absorbed from a low concentration in air by harvested green, but mature, fruits, many ripen faster than normal. Although ethylene has been used commercially for many years to ripen fruit, its mechanism is unknown and is being investigated. With the avocado as an experimental subject, the complicated ethylene chemistry was studied. Carbon and hydrogen from ethylene enter into different series of chemical reactions in the fruit. About one-eighth of the total hydrogen was incorporated in the methyl group of toluene, but only a minute amount of the carbon ended in toluene. A smaller amount of the ethylene was found in another hydrocarbon, benzene. The proportion of original ethylene, carbon, and hydrogen found in toluene and benzene suggests that these two end compounds may be products of separate reaction chains and not successive steps in a single chain of reactions.

Biochemical Synthesis of Structural Material. Enzymic synthesis of structural plant tissues, especially the wall substance of plant cells, is being investigated. The properties of such tissues mainly determine such textural

characteristics as crispness, turgidity, and ability to maintain structural integrity through boiling, freezing, or drying. These enzyme systems can only be investigated in growing tissue, but growing tissue always has accidental variations. Two laboratory techniques are used to get more uniform working materials. One technique involves growing plant tissue cells in a complete synthetic nutrient solution. Another method is to use newly-formed plant tissues in oat seedlings that have been germinated and grown under very uniform conditions. When the amino acid proline was added to the solutions of plant tissue cultures, it was quickly incorporated into peptides and proteins that form a part of new cell walls. At the same time hydroxyproline, which forms by enzymic oxidation of proline in air, appeared substantially in the cell wall proteins but very little in the protoplasmic protein within the cell. Protoplasmic protein incorporated proline very rapidly, however. In the cell wall the two amino acids are apparently firmly associated with carbohydrate materials such as pectin, hemicellulose, and cellulose, which are mainly responsible for the mechanical properties such as stiffness and elasticity. Application of a potent growth promoter, indolacetic acid, to the live oat seedlings weakened their wall structure. Enzymic removal of pectins or various proteins from the seedling walls did not further weaken them, indicating that the strength is mainly provided by hemicellulose and cellulose material.

Enzymic Mechanism for Replication of DNA. Special enzymes in growing plant tissue are involved in reproducing the genetic pattern in new cells of growing plants. Desoxyribonucleic acid (DNA) duplicates itself under proper conditions. Research is conducted to discover how this replication of DNA is modified if DNA is complexed with histones, a class of proteins rich in either arginine or lysine which readily associate with DNA molecules. In a cell-free test tube system, replication of DNA molecules proceeds if a DNA-histone complex is used instead of DNA. However, the synthesis proceeds slower. In such experiments the DNA-histone complex can be either a natural product, such as one that has been separated from pea embryos, or it can be synthesized from certain purified proteins and DNA. In either case the DNA will be replicated. When a lysine-rich histone was used, only a little new DNA forms, but if an arginine-rich histone was used, somewhat more DNA appeared. Many plant physiologists wonder how tissues of a plant can arise from a single cell but manage to differentiate from one another and produce entirely different tissue structures at different locations and at successive stages of the life cycle. A current hypothesis identifies the mechanism that accomplishes this differentiation with the histones that complex with DNA. The DNA replication system and the enzymes that monitor it are being explored.

Plant Protein Synthesizing Enzymes. The process of protein synthesis in a plant consists of the orderly assembly of all the amino acids required to form the protein and their attachment, one after another or in groups, to build up structures whose pattern is specified by ribonucleic acid (RNA) molecules in the plant cells. Synthesis is accomplished through reactions whose first step involves activation of an amino acid molecule by a specific enzyme and a nucleotide triphosphate to furnish necessary energy. A

polymerizing enzyme then attaches the activated amino acid to the emerging protein structure. The reaction in which the activated amino acid-phosphate-enzyme complex is associated with a molecule of RNA is under investigation. A strong solution of purine disrupts the helical secondary configuration of RNA that is believed to be associated with the specificity of this molecule for association with a particular amino acid. We don't know whether the destruction of this helical arrangement interferes with the ability of RNA to associate with an amino acid. Research to date demonstrated that the reaction that activates an amino acid is not affected by the presence of purine. The effect of purine on the attachment of amino acid to a protein chain will now be studied.

Biochemistry of Plant Steroids. Steroids sometimes control enzymes. Steroids and related compounds are being investigated in cooperation with the Division of Biology at the California Institute of Technology. The major steps in biosynthesis of certain steroids from acetate to diosgenin were identified by administration of radioactive tagged precursors to growing plants or plant tissue homogenates and isolating radioactive products by thin-layer chromatography. A number of chemical compounds known to accelerate or retard particular enzyme reactions were studied to determine the nature of their action on the plant production of growth stimulator. A tagged steroid precursor both with and without added retardants was added to the fungus Fusarium. Radioactive gibberellin, an active growth promoter, was isolated from retardant-free cultures but was not found in cultures containing the retardants.

Chemical Alteration of Enzymes. Chemical modification of pure crystalline enzymes in order to locate active regions in protein molecules and to determine relationship of molecular structure to enzymic activity is being investigated by a collaborator with the Pioneering Research Laboratory whose research is supported in part by a grant from the National Institutes of Health. This investigation uses isolated proteins which combine with enzymes to inhibit enzymic action. A protein was isolated from the potato and determined to be a very potent inhibitor of the protein-splitting enzyme, chymotrypsin. The inhibitor, the enzyme, and the complex of the inhibitor and enzyme have all been crystallized. The complex is inactive either as an enzyme or an inhibitor, but it was possible to disrupt the complex and resolve it into active enzyme and active inhibitor. The complex contained four molecules of the enzyme per molecule of inhibitor. This is the first known instance of a combination of a proteinaceous inhibitor with a protein-splitting enzyme in any ratio but 1:1. Such knowledge should be helpful in determining the active center of the enzyme. The purified chymotrypsin inhibitor is used in eye surgery. Chymotrypsin has been effective in the treatment of cataract, but a safe way of stopping the protein-splitting action when desired was needed. The new inhibitor makes this possible and is not toxic in the quantities required for eye surgery. The effect of changing the structure of chymotrypsin by chemical reaction was also studied. Others have shown that trypsin, another protein-splitting enzyme, loses its ability to complex with proteinaceous inhibitors after acetylation but

acetylated chymotrypsin was inhibited by the potato juice inhibitor just as plain chymotrypsin. This result is another clue to the active center of this enzyme.

B. New and Improved Food Products and Processing Technology

1. Dried Fruit Products. Research on new and improved dried fruit products continues, supported in part by the Dried Fruit Industry Research Advisory Committee which provides the salary of one of the scientists assigned to this work. A dried fruit product suitable for inclusion with dry breakfast cereals was prepared from prunes, dried figs, or dried apricots. Dried fruits were ground to a paste, dried on a double drum dryer to a desired moisture content, ground into a powder, and pressed into suitable size disks for inclusion in breakfast cereals. Adjustment of drying conditions and tableting pressure, produces disks dry enough that they do not lose moisture to the cereal and crisp enough to keep an attractive texture for a suitable period after being immersed in milk with the cereal. Samples of this product were supplied to cereal manufacturers for evaluation.

A modification of drum drying has produced a good apple sauce flake, both sweetened or natural, smooth or chunky. Air is drawn over the drying film on the revolving drum surface and then a chilled air blast hits the film just before it is scraped from the drums. The method is now in use by a processor in Washington State and his product is being used by cake mix manufacturers. The product has also been adapted for the Apollo space feeding program.

Heat treatments to prevent setting of raisin paste were simplified by heating whole raisins in either hot air or steam prior to grinding, and packaging the ground raisins while still hot. The treated raisin paste remained soft and pliable during prolonged storage and is, therefore, useful in bakery formulations.

2. Fruit Dehydration. Microwave heat treatment for cut fruits accelerated subsequent dehydration. The heat inactivation of enzymes made possible a reduction in sulfite addition to such fruits. Microwave heating was also used to sterilize flexible packages of fruits that were packed in the moisture range between 35% and 85%. Further advances were made in osmotic drying of apple chips. Thin apple slices, dipped in salt-citric acid solution, were mixed with sugar and held for several hours or more. Moisture passed from the fruit to the sugar turning it into syrup, which was drained. The product was then dried for two to three hours in a vacuum oven with a high temperature source (212° F.). The apple chips so produced were light in color although no sulfur dioxide had been used, and had a good apple flavor. The texture was crisp because of the rapid vacuum drying to a moisture content of about 1%. Although this product is stable against non-enzymatic browning, its resistance to oxidative deterioration presents a problem for future research. Syrup formed from the sugar and fruit moisture in this osmotic drying process can be used for flavor concentrate and sweetener in the manufacture of experimental ice cream, or in an integrated apple processing plant it could be used for canning syrup and for canned apple sauce.

Microwave energy has also been used to puff partly dried apple sauce to insure rapid final drying and ready rehydration. In this method, apple slices are degassed in vacuum and the vacuum is broken with a mixture of CO_2 and SO_2 gases. The slices are dried to 20% moisture in warm air and treated with microwave to remove 1 to 2% moisture, thereby puffing the product which is then air dried to its final moisture content.

A gelled apple sauce product was developed so it can be used on a warm plate without losing its form, used for molded salads, etc. The product is formulated with low methoxyl pectin and calcium salt to harden the gel. Commercial market tests are currently under way.

Texture of Processed Fruits. No large-scale softening of 1964 brined cherries was reported on the West Coast, perhaps partly because of the results of cooperative studies conducted with Oregon and Washington State Agricultural Experiment Stations over a several-year period. Increasing numbers of brined cherry processors used the puncture meter as a quality control instrument as developed by the Western Utilization Research and Development Division to indicate possible softening early when the deterioration could be arrested by drawing off the brine, heating it, and then adding it back to the cherries. High calcium brines have also been used as insurance against texture deterioration.

Studies were conducted on the reuse of old brines. Cherries processed in brines reused several times were firm and had acceptable texture. Anthocyanin build-up in the brine could discolor brined cherries in storage if all the natural pigment was not bleached out prior to dyeing the cherries. Heavy metal ions such as copper, iron, and manganese in amounts over 5 p.p.m. contribute to the formation of undesirable color. Reuse of calcium bisulfite brines for more than one season reduces costs of cherry brining not only in purchase of supplies but also in the more serious problem of disposal of waste brines.

4. Grape Juice and Grape Products. Research on viniferous grapes has been extended to studies of wine technology with a small in-house research effort supplemented by informal cooperation with commercial wine makers and by contract research. Wine grapes invaded by the mold Botrytis cinerea in the vineyard or inoculated by mold spores sprayed on harvested grapes in a controlled environment yield wines generally considered greatly superior to those from uninfected fruit of the same variety. The better Sauternes from France, Tokayi Essencz, Passito de Caluso, and Edelfaule Auslese are such wines. Investigations have been centered on the novel approach of fermenting grape must (crushed grapes and juice prepared as a first step in wine making) or juice with Botrytis rather than allowing it to grow on the whole fruit. For a successful controlled fermentation of this type, competing molds and micro-organisms must be controlled to allow the Botrytis organism to predominate. Heat sterilization or pasteurization harms flavor and color so non-thermic microbial control is used. Diethylpyrocarbonate, sulfur dioxide, and benzoate of soda are three sterilants that have been used experimentally.

Beneficial effects have been obtained by submerged culture of Botrytis cinerea and by addition of Botrytis cinerea mycelium extracts to the must or the grape juice. Pectin-splitting enzymes from the mold make the wine easier to filter. Most important, Botrytis fermentation improved the flavor substantially and wines made from Thompson seedless juice (characterized as rather flavorless) show considerable similarity to French Sauternes. Moderate scale wine improvement experiments were conducted cooperatively in two commercial wineries. However, for the first year's experimentation bonification of wine varietal grape rather than Thompson seedless grapes were used. Larger-scale experiments and experiments with Thompson seedless grapes are anticipated during the next year.

Osmotic concentration of grape juice and wine was accomplished without application of heat by drawing water from wine into a concentrated salt solution through a dialysis membrane. Preliminary studies with various dialysis membranes indicate the possibility of removing alcohol from wine without using heat but improved membranes are probably necessary. The product would be a low-alcohol or nonalcoholic beverage with wine flavor.

Effects of diethylpyrocarbonate as a prefermentation sterilant for alcoholic fermentation, in addition to its use in studies of the Botrytis cinerea treatment, are under continuing investigation. The use of this apparently harmless compound which hydrolyzes rather rapidly into ethanol and carbon dioxide could obviate or reduce the use of sulfite in white wine production and minimize the sulfhydryl type odors and residues of sulfur dioxide in such wines.

Contract research was initiated at the University of California at Davis on new or improved methods for separation of juice from grape pulp, skin, and seeds (must). Flash steam heating of red grapes to improve color extraction from skin was studied from the standpoint of its effects on aroma. Gas liquid chromatography showed that certain esters (isoamyl acetate and ethyl caproate) develop to higher levels in heat-treated juice. Recovery of fermentable sugar from grape pomace was also investigated under this contract. Countercurrent washing of pomace appeared to provide a satisfactory recovery of sugar.

Contract research was initiated at the New York Agricultural Experiment Station in Geneva on the chemistry of undesirable precipitates that form during the production of wine in order to develop improved processes and fining material to remove haze and precipitates from wines.

5. Processing Quality of Varieties of Northwest Fruit and Berries. Fruit and berry selections are evaluated in cooperative research with the Washington State Agricultural Experiment Station. Six varieties and 21 hybrid selections of strawberries were evaluated. The hybrids represent the best of 10,000 seedlings which first produced fruit in 1960 and, although none were found to possess processing and cultural characteristics that justified introduction as new varieties, several are promising from some standpoint and are

being kept by the geneticists at the Experiment Station for breeding stock. Earlier evaluations on a new hybrid for preserves played a part in its release in Oregon as the Banks strawberry variety. In cooperative work with Washington, Oregon, and California Agricultural Experiment Stations frozen strawberries were compared for processing quality. Processing evaluation of 18 hybrid selections of raspberries indicated one hybrid is outstanding. It will now be propagated for introduction as a variety in the State of Washington. These resistant hybrids should fill an existing need where present susceptible varieties are declining.

Preliminary evaluation of processing quality of pears was undertaken to determine whether or not faults had been introduced into new strains being developed for simplifying mechanical harvest. For example, introduction of dwarfing root stocks, plant hormone sprays for shortening pear tree internodes, and gibberellin sprays for setting pear fruits after severe frosts during bloom. First-year evaluation indicated hard end disorder and offset stems occurred frequently. Gibberellin sprays caused misshapen fruit and abnormal cores. Observations must be continued during several growing seasons to be significant.

6. Improved Fruit Juices and Fruit Juice Processes. An improved apple juicing system, in which a conventional rack and cloth press was replaced by a sanitary basket centrifuge and a stainless steel continuous vertical screw press, was developed and used in test runs at commercial apple processing plants in northern California and Washington. In most cases the yields of juice and percentage of solids in the juice are both significantly better than those from a rack and cloth press. Gross juice yields range from 160 to 190 gallons per ton of fruit, depending upon maturity and variety. Addition of a press aid before centrifuging was essential. The system lends itself to full automation and is best suited to large-scale commercial operations because of sizable capital investment involved. Following publication of results an apple processor in British Columbia installed the system.

Engineering studies to improve methods of transferring heat to fruit juices and purees during processing have centered on factors affecting the fouling of heat transfer surfaces. Because rotating steam coil heat exchangers have become widely used in open pan evaporation, particularly for tomato juice, equipment was fabricated to initiate studies on fouling of such heat transfer systems. In preliminary tests, steam condensate in the coil resulted in an uneven temperature gradient which affected the experimental values, and a modified rotating steam coil is being designed for further work. Control of heat input for evaporating food materials is hard because juices or purees entering the system are not uniform. To compensate for variable total solids entering an evaporator, an automated feed forward system is being developed. In continuous operation the total solids of juice entering the evaporating system would be sensed and used to activate the heat input as the juice entered the evaporator. A mathematical model using a realistic variety of operating parameters was developed simulating a feed forward system on an

analog computer. The feasibility of the system was proven and the next step will be to prove out the control under operating conditions. Two in-line optical refractometers to determine total solids of input juice and outflow concentrate and an analog computer will be installed in a long tube evaporator and experiments conducted to confirm the mathematical simulation.

7. Stabilizing Shelled Nuts. Deterioration of shelled nuts is presumably due to the oxidative changes in unstable lipids. Research was initiated on the analysis of walnut oil, and a modification of laboratory procedures to facilitate this analysis was successful. It involved a direct, rapid, convenient, and quantitative transesterification of walnut oil glycerides using a boron trifluoride catalyst. The method should be applicable to similar studies with other fats and oils.

Contract research on the stability of fresh and roasted Macadamia nuts at the University of Hawaii was terminated. The final report revealed that only minor differences were observed in quality and chemical characteristics of three varieties used. Roasted kernels maintained their quality better than raw kernels. Flavor deterioration closely paralleled darkening of kernels. Kernel stability increased with decreasing moisture content down to about 1% moisture. Kernel stability decreased with increasing storage temperature. Quality changes were essentially identical for kernels stored in light or darkness. Satisfactory conditions were found for processing and packaging shelled Macadamia nuts that are stable.

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AREA NO. 7. POTATOES--PROCESSING AND PRODUCTS--WESTERN LABORATORY

Problem. The potato industry, faced with a continuing decline in the consumption of fresh potatoes, is becoming more and more dependent upon the development of new and improved processed products to maintain markets and to avoid recurring economic disasters. Crop perishability, supply fluctuations, and the inelasticity of demand, result in wide swings in price with even slight surpluses. In producing areas having a substantial processing industry, depressive lows are moderated by advance contracting by processors prior to harvest. However, in many important potato growing areas processing has not yet developed, and vulnerability not only still exists, but is exaggerated by the growing competition of processed potato and other competing food products. A continuing improvement in processed potato products is clearly required if processing is to expand fast enough to offset the progressive decline in use of fresh potatoes.

To improve the quality of processed potatoes, ways must be found to eliminate the stale, earthy, rancid, green, and warmed-over flavors that are sometimes encountered in potato products, including dehydrated mashed potatoes, dehydrated diced potatoes, frozen French fries, frozen patties, and potato chips. Equally important, methods must be devised to retain the desirable natural flavor of the freshly cooked potato in the processed product. Recently developed research methods offer an opportunity to isolate and identify the constituents responsible for the natural flavors and the off-flavors, to develop rapid and sensitive analytical methods for their measurement, and to determine the raw material factors controlling formation of the various desirable and undesirable constituents in the fresh potato. Further improvement in the texture of potato products is also needed. Fundamental histological and chemical investigations could be used to determine the causes of differences in the texture of potatoes, as a basis for developing improved processing methods. Enzymes play a great part in the entire compositional pattern of the potato, not only the constituents responsible for flavor, off-flavor, color, and texture, but also those responsible for disorders such as black spot. Black spot causes severe losses both to those who market potatoes fresh, and to those who process potatoes, because trimming costs are sharply increased and yields reduced. Increased knowledge of enzymes is needed as a basis for solution of the black spot and similar problems, to increase use of potatoes by reducing costs, and to improve quality of both fresh and processed potatoes.

USDA AND COOPERATIVE PROGRAM

In the Western Utilization Research and Development Division, basic and applied research on potato products is conducted at the Division headquarters at Albany, California, and by grant funds under P.L. 480 in

England and Sweden. The chemistry of potato flavor and the compounds involved in deterioration of potato products are studied to provide a basis for new and improved potato processes and products. Histochemical studies are conducted to elucidate factors involved in the texture of potato products. Basic investigations on the enzyme systems involved in potato product discoloration and the mechanism of rancidity development are in progress.

The Federal program of research in this area totals 5.8 professional man-years. Of this number, 3.1 are assigned to chemical composition and physical properties and 2.7 to new and improved products and processing technology. In addition, the Division sponsors two research grants under P.L. 480 on basic studies.

PROGRAM OF STATE EXPERIMENT STATIONS

State stations have a continuing long-term program of basic and applied research related to potato utilization. These studies cover the spectrum of problems ranging from the effect on quality of production practices such as variety, fertilization and management, to shelf-life, quality and flavor of processed products.

Mechanical harvesting procedures and storage conditions affect potato composition and in turn suitability for processing. Relating variety, raw material characteristics and storage conditions to quality of the various forms of processed potatoes received attention at a number of stations. Much effort continues to be given to careful composition studies, particularly those relating to nitrogenous constituents, lipids, amino acids and sugars.

The potato industry is becoming more and more dependent upon development of new and improved products to maintain per capita consumption levels. Product research is directed to solution of problems of texture, sloughing, discoloration and processing procedures such as deep fat frying.

Newer techniques of flavor research are being applied in the search for an understanding of the flavor and flavor changes in potato products. For example, the volatile flavor compounds associated with fresh and stale potato chips are being investigated in order to better understand the changes which occur in these compounds during storage.

The utilization program also includes studies designed to find new and improved uses for sweet potatoes. Studies related to determining suitability for canning, freezing and dehydration are continuing. New research is related to developing improved methods for drum drying sweet potatoes and for drying purees of high solids content. Problems of packaging, additives, storage stability and preparation and use also are under investigation.

The number of professional man years devoted to potato utilization research is 9.4.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Flavor Stability. Oxidative deterioration is the predominant storage problem of dehydrated mashed potatoes. Although most food products can be stabilized by refrigeration, oxidation of potato granules and potato flakes continues even at 0° F. Gas liquid chromatography was used to analyze the vapor above reconstituted potato granules after controlled storage tests. Taste panel judgments of flavor correlated well with the hexanal content of the vapor. Deterioration was distinguishable by judges after air-packed granules had been stored three months at 75° F. By that time the hexanal concentration had increased more than tenfold. During the next month, hexanal increased fourfold more. Hexanal appears to be a useful index of oxidative deterioration, but it should not be thought that hexanal is mainly responsible for the characteristic flavor of oxidized granules. Other compounds produced in fatty acid autoxidation almost certainly contribute also.

Basic research on the autoxidation of fats in dehydrated vegetables is conducted under a P.L. 480 grant at the Swedish Institute for Food Preservation Research in Gothenburg, Sweden. Autoxidation can be catalyzed by divalent copper ions. The rate of chemical reaction was measured in model systems consisting of linoleic acid emulsions which were subjected to various conditions of oxygen concentration. Lowering the oxygen concentration reduced the rate of autoxidation, and the reduction was more pronounced in the presence of the copper ions than in the absence of copper. A nearly complete removal of oxygen from the system was necessary to reduce the autoxidation rate by 50%.

2. Enzymic Browning. The nature, distribution, and mode of action of enzymes responsible for browning of potatoes and potato products are studied under P.L. 480 grants at the Low Temperature Research Station in Cambridge, England. Extensive experiments were conducted on the complex influence of soil and climate on browning and tyrosine content of potatoes. (Tyrosine is a component of potatoes that turns to a reddish brown when oxidized under the influence of enzymes.) Urea added as a nitrogen fertilizer increased the amount of tyrosine in potatoes, whereas chloride depressed it. The greater the water content of potato tubers the greater was the concentration of tyrosine. To study the mode of action of phenolase enzyme, it was extracted from the horse bean, a potent source. Comparative studies were made of phenolic substrate biosynthesis in tomato plants, which are closely related to potatoes. The role of minerals in phenolic biosynthesis was mainly an effect on carbohydrate metabolism. Diurnal variations were observed in caffeic acid and sugar concentration in tomato leaves. Caffeic acid increased markedly in the first four hours of darkness and then declined for the rest of the night. During the day, the level either increased or remained constant. Soluble sugar concentration decreased slowly at night, increased sharply during the first two to four hours of light, then decreased during the remainder of the day.

B. New and Improved Products and Processing Technology

1. Effects of Processing on Product Stability. Over 20 years ago, British scientists established that high moisture levels in potato granules retard oxidation, and the addition of sugar to potato granules increases their resistance to browning at high temperatures. We have initiated storage studies of high-moisture (12-13%) granules with sugar added. If the addition of sugar can successfully prevent browning, we may be able to develop dehydrated mashed potato that is stable without gas-packing or antioxidant.

Frozen french fried potatoes for institutional and restaurant use make up one of the largest, if not the largest, of the commercial frozen vegetable packs. This product is widely used in small restaurants without adequate freezer space to hold the potatoes until they are used. We have made bacterial counts and evaluated the flavor, texture, and color of frozen french fried potatoes held at common refrigerator temperatures (34°, 45°, and 55° F.). Off-flavor was detected in about a week at 45° F., and bacterial counts rose substantially. However, no health hazard was indicated. Most of the bacteria were killed in the finish frying, as practiced in restaurants using this product. After four days' storage at 45° F., bacterial counts of commercial samples of frozen french fried potatoes did not exceed 100,000 per gram, a count that is allowed in Canadian regulations for frozen foods and that has been suggested by some health authorities as a suitable standard here.

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AREA NO. 8. VEGETABLES--PROCESSING AND PRODUCTS--WESTERN LABORATORY

Problem. Vegetable crops, in general, are perishable and seasonal and thus are subject to supply and price fluctuations to the disadvantage of the agricultural economy. In order to expand markets and stabilize prices, new and improved processed products are needed that will be more desirable to the domestic and foreign consumer from the standpoint of quality, convenience, stability, nutritive value, safety, and cost. The quality of processed vegetables and the economy of their processing have not improved rapidly enough to increase or even maintain the relative position of vegetables in the American diet, or to increase substantially their contribution to the export trade. The consumption of dry beans and certain other vegetables is limited by the fact that they cause flatulence.

New easy-to-prepare vegetable products are needed, particularly from such commodities as dry beans and peas, which now require hours to prepare. The severe heating required to sterilize low-acid foods, which include most vegetables, seriously impairs the quality of canned products. The stability of all kinds of processed vegetables needs to be improved so that quality and nutritive value will be better preserved during storage and distribution. The safety and effectiveness of new chemical additives, needed to improve the quality and stability of processed vegetables, must be established. Better methods of removing residues of agricultural chemicals from vegetables for processing are urgently needed, as are procedures for decontaminating vegetables exposed to radioactive fallout. Of vital importance is research to reduce the costs of processing in order that the farmer may receive a larger share of the consumer's dollar.

Applied research on these practical problems must be supported by a strong program of basic research on the chemical constituents of vegetables responsible for flavor, color, and texture; on the reactions these compounds undergo before, during, and after processing; on constituents having biological activity; on the microsopic structure of vegetables and vegetable products; and on the micro-organisms which cause spoilage or loss of quality in these products.

USDA AND COOPERATIVE PROGRAM

In the Western Utilization Research and Development Division, a broad program of basic research on vegetables and the application of science to new and improved products and processes is conducted at the Division headquarters at Albany, California, in field stations at Pasadena, California and Puyallup, Washington; by contract at Urbana, Illinois, East Lansing, Michigan, and Davis and Berkeley, California; by a grant at Urbana, Illinois, and by grants under P.L. 480 in Finland, India, France, and Sweden. Fundamental studies are conducted on the chemistry of vegetable

flavor and vegetable pigments, the mechanism of heat resistance in bacterial spores, the composition of dry beans as related to cooking quality and flatulence-producing characteristics, the factors affecting deterioration of dehydrated vegetables, and the microbiology of raw vegetables for processing. Applied research is conducted to develop new and improved products to increase the utilization of vegetables including new, high quality concentrated and dehydrated products and products of improved convenience of use, processes for producing these, improved freezing methods, selection of improved processing varieties, and methods for removing radioactive fallout.

The Federal program of research in this area totals 33.3 professional man-years, including four scientists whose salaries are provided by the California Lima Bean Advisory Board operating under a State Marketing Order and the United States Brewers Association; and six contracts and grants equivalent to approximately 4.6 professional man-years per year. Of the total, 20.7 are assigned to investigations on chemical composition and physical properties and 12.6 to new and improved food products and processing technology. In addition, the Division sponsors four grants under Public Law 480 on basic research.

PROGRAM OF STATE EXPERIMENT STATIONS

State stations conduct a broad program of basic and applied research on vegetable processing and products in order to maintain the place of vegetables in the diet and to overcome problems associated with the perishability and seasonality of vegetable crops. Research on the adaptability and evaluation of vegetable varieties for processing is a continuing service to vegetable breeding programs. Each promising introduction or variety is evaluated with respect to processing yield and characteristics.

Increased public concern with protection of the food supply from pesticide residues has resulted in initiation of three regional projects to study reduction or removal of residues from food products. Since commercial food processing and preparation procedures vary, the effect of these processes on residue removal is being evaluated. There is also an urgent need to develop rapid, sensitive methods for routine determination of pesticide residues on foods undergoing commercial processing. Data relative to chemical form, distribution and persistence is being amassed. Vegetables are included in the crops being studied. One objective of regional project NEM-30 involves study of the basic physiology and chemistry of changes taking place in post-harvest handling and processing.

Characterization of raw materials extends to consideration of the effects of various production variables upon processed product quality. Mechanical harvesting and the associated effects upon ultimate processed product quality are receiving increasing study. The degree of correlation or association between color, flavor and texture in fresh and in processed items continues to be a major concern.

Basic chemical and physical properties of vegetables are related to product acceptance and quality. Research on vegetables in this area ranges from standard composition studies to highly specialized analysis for mineral components. Research aimed at describing the biological changes that occur in vegetables at different stages of maturity continues. The role of enzymes and pigments in vegetables also receives continuing study.

Basic microbiological research centers around the high resistance of bacterial spores to heat and the adverse effects extreme thermal process requirements have on canned vegetables. Microbiological studies extend from determination of thermal process requirements to study of the natural flora of fresh vegetables. The radioresistance of bacterial spores and use of combined antibiotics and heat are carefully researched.

Processing technology research is directed to studies of freeze-drying, brining, canning, fermentation, hydro-cooling and controlled atmosphere methods. The comprehensive study of the effects of controlled or modified atmosphere on the biochemical, physical and quality characteristics of various vegetables continues.

New or improved product development research seeks to improve or perfect such items as "quick cooking" peas and beans, beet chips, various snack items; and new sauerkraut products. Basic information relative to composition, nutritive value and functional properties is emphasized.

The total station research effort devoted to vegetable processing and products is 64.1 professional man years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Vegetable Flavor Components. Basic investigations on vegetable flavor are centered on the chemistry of volatile components and include research on tomatoes and onions. In addition, with support of the United States Brewers' Association, which has provided the salaries of two chemists, compositional studies of hop oil are conducted.

In concentrations of about 1 part per million, a few hundred times the olfactory threshold, methyl sulfide contributes to the aroma of canned tomato products. Samples having one-half to one p.p.m. were judged superior to those having lower or higher concentrations. S-methyl methionine sulfonium salt was isolated from fresh tomatoes. The mechanism of its thermal decomposition to yield homoserine and methyl sulfide was confirmed; now it must be considered an important flavor precursor for cooked tomato. Mass spectroscopy has been very useful in identification of extremely dilute organic compounds. A series of butyric and hexanoic esters of 2-, 3-, 4- and 6-carbon alcohols were prepared and tagged with heavy hydrogen. Mass spectral

decomposition patterns of the compounds were determined to aid in identification of unknown esters.

An amino acid isolated from dehydrated onions by a collaborator from California State Polytechnic College was found to be propenyl cysteine sulfoxide, which had previously been identified by Nobel Laureate, A. I. Virtanin, a P.L. 480 grantee in Finland. This amino acid is the precursor of the substance in onions that makes us shed tears. It was identified by carbon hydrogen analysis, infrared spectroscopy, specific optical rotation, and isomerization with base to cycloalliin.

Related research on organic components in vegetables and fodder plants is supported by a P.L. 480 grant to Prof. Virtanin at the Biochemical Institute in Helsinki, Finland. Particular attention is given to isolation and identification of sulfur compounds in onions and enzymic and chemical decomposition compounds that have strong flavors or lachrymatory effects.

Our research on hop oil is aimed at isolating and characterizing volatile components that may contribute to flavor of hops. Eighty-seven constituents were separated from the oxygenated fraction of hop oil; forty-two were identified by comparison of mass spectral patterns and gas chromatography retention times with those of authentic samples. Thirty-five other volatiles were detected by their mass spectral patterns and by other analyses. Odor thresholds of 30 major hop oil constituents in water solution were determined. These results were confirmed by preparation of a reconstructed hop oil prepared combining 27 components in the proportions found in natural oil. A number of experienced judges found the mixture was quite similar in aroma to natural oil. A detailed gas chromatographic analysis of 13 American and European hop oils classified them into several rather distinct groups. Such a classification will be valuable to brewers because hop varieties are not readily distinguishable by other methods.

2. Nature of the Heat Resistance of Bacterial Spores. Flavor, color, texture, and nutritional quality of canned vegetables deteriorate under the sterilizing heat requirements necessary to destroy bacterial spores. Year by year the nature of spore heat resistance is being revealed. Although a complete explanation of the mechanism is not yet available, basic observations have taught us how to activate spores or restore them to dormancy and to reversibly control their resistance to heat. These can now be done by chemical means rather than by the use of heat. Spores of B. subtilis were found to have a cation exchange system. The heat resistance of the spores depends on the cations with which they are loaded. When hydrogen ion, for example, replaces calcium in this system, the spores lose resistance to heat. This exchange accounts for the long recognized lability of spores to heat under acid conditions. The heat-sensitive hydrogen form may be made resistant again by loading the spores with calcium again. These simple chemical treatments are similar to regenerating and using a water softener. Another spore phenomenon, heat adaptation or acclimatization, was found. Moderate heating increases the resistance of spores to greater heat when the heat-

sensitive hydrogen form is in a buffered solution of divalent cations. The rate and temperature characteristics of the hydrogen-calcium exchange are similar to the heat adaptation of bacterial spores and may control it. The controlled modifications in heat resistance involve no growing of bacterial cultures. These chemical treatments to effect heat adaptation can be reversibly imposed upon resting spores. Preliminary laboratory studies with vegetable purees have demonstrated the possibility of reducing by 50% the heat treatment required to sterilize products containing large inocula of spores of B. stearothermophilus, B. subtilis, and Clostridium #3679.

Our earlier observations strongly suggest that the heat resistance of spores is related to their density. Neutral lead chelates were prepared in aqueous solutions and used for a density gradient separation of spores. Most of the spore samples used could be separated into more than one band under the density gradient. Separating spores of different density from the same culture appears to be a useful research approach.

A research grant was made to the University of Illinois to provide basic information on the mechanism of spore formation in anerobic bacteria that spoil food and to develop laboratory techniques for producing plentiful samples of spores of high and uniform heat resistance. Such samples would facilitate research on the heat resistance of spores in canning low-acid foods. The biochemistry and physiology of thermophilic anaerobes is obscure primarily because they are hard to culture. Observations on the mechanism of heat resistance of obligate anaerobes will be compared with the exchangeable cation control of heat resistance that we have discovered with spores of aerobic species.

Investigations supported by a P.L. 480 grant to the National Institute of Agronomic Research in Paris were initiated to identify those enzymes essential for spore germination that are inactivated only by severe heat, and to isolate and characterize these enzymes. Moderate heating of bacterial spores stimulates spore germination but severe heating inhibits it. Metabolic activity during spore germination is under investigation, including the nature and property of enzymes that are involved in spore germination and the exact stage at which the germination of severely heated spores stops. These results will be extended to spores of bacteria that are important in the heat processing of vegetable and other low-acid food products. Others demonstrated that amino acids used during spore germination or vegetative growth are probably metabolized by an amino acid dehydrogenase system which deaminates amino acid. Our grantee in Paris studied the enzyme system involved with material extracted from vegetative cells. Two amino acid dehydrogenases were observed; one was an enzyme not previously described. No apparent correlation was found between the effect of amino acids on spore germination and the properties of these amino acids as substrates for enzyme activity.

3. Composition of Dry Beans. We have reduced the search for the flatulence-producing agent in beans to a fraction which is insoluble in ether, soluble in 60% ethanol, passes with the smaller molecules through a dialysis membrane

into distilled water, remains in solution in 85% ethanol, and is not retained on a cation exchange column. The major flatulence-producing activity is thus in a mixture of low molecular weight compounds constituting about 6% of the initial dry bean solids. In the process of this separation, a way was found to recover from beans more than 80% of their protein in the form of a bland, colorless, flatulence-free powder. Galactose-containing oligosaccharides such as are found in the flatulence-producing fraction increased breath hydrogen, suggesting increased bacterial fermentation in the intestine. The egestion of hydrogen as flatus appears to depend on the subject's respiratory efficiency. Exercise significantly reduced the amount of flatus following a bean meal, probably because of increased ventilation of the lungs. Administration of the drug Vioform, a bactericide, with an experimental bean meal decreased the expected volume of hydrogen, increased the volume of methane, and had little effect on the volume of carbon dioxide in the resulting flatus. Measurements of physiologically inert gases, argon and nitrogen, in flatus by mass spectrometry indicated that nitrogen in flatus arises from swallowed air. In contract work at the University of Illinois experiments are underway with anesthetized dogs. These studies may demonstrate the role of carbonic anhydrase in the transport of carbon dioxide through intestinal walls and inhibition of carbonic anhydrase. However, bicarbonate secretion from the pancreas is not the only source of carbon dioxide of flatus when beans are consumed. Sterilization of the small intestine with antibiotics did not prevent production of gas in that area.

4. Cookability of Beans and Peas. A basic investigation on the influence of phytin on the texture of dry peas during maturation under P.L. 480 at the Fruit and Vegetable Canning and Quick-Freezing Research Association Laboratories at Chipping-Campden in England was concluded. Cooking quality of dry peas is variable; some batches become too soft and others remain very hard after a standard process. Harvesting and drying conditions did not alter the texture of dry peas, but storage at relative humidities over 60% and at temperatures above 70° made dried peas harder to cook. Extensive studies on the phosphorus content and phytic acid of peas indicated that peas with less than 75% of the total phosphorus content as phytate were hard after a normal cooking process, but high phytate content did not guarantee good cooking quality. High calcium in the cell walls of cooked peas correlated closely with toughness; phytin removed calcium by forming insoluble calcium phytates during cooking. However, added phytic acid did not wholly prevent toughening because even in high concentrations of calcium only 60% of the phytate formed complexes. A high phytate content ameliorated the effect of added calcium or magnesium. Storage under unfavorable conditions did not change the distribution of calcium and magnesium in the peas. Textural changes are thought also to be related to modifications in the starch. A decrease in the solubility of starch in perchloric acid and a decrease in starch specific gravity were observed in storage of peas under adverse conditions.

An investigation of the proteins, amino acids, and biologically active components of dry beans was initiated under P.L. 480 at Allahabad University,

Allahabad, India. Procedures for extraction of protein were developed and applied to Phaseolus vulgaris and Phaseolus munga. Semiquantitative fractionation and isolation of proteins from these beans by paper electrophoresis techniques is underway.

5. Vegetable Pigments. A simple microtechnique was developed for the detection, identification, and proof of purity of various chlorophyll components in very dilute extracts from green vegetables. The use of powdered sugar as an adsorbent for thin-layer chromatographic separation of chlorophyll components was an improvement over paper or column chromatographic separations. Chlorophyll degradation products, pheophytin a and pheophytin b, were separated by ascending thin-layer chromatography and could be observed and photographed under ultraviolet light. Fluorescent areas were clearly visible and could be marked, scraped from the plate, and eluted from the sugar for further identification.

6. Histological Studies of Vegetable Tissue. Microscopic studies with green beans indicated that it is possible to freeze beans rapidly enough to prevent visible damage to the tissue and that these beans have firmer texture than those frozen more slowly. Because the usual techniques of the microscopist are too tedious for routine processing control, a new method was developed for examining frozen beans to determine visible tissue damage. Frozen beans to be examined were placed in methanol at about 0° F. Without melting, the water dissolved and was replaced by methanol. Thin slices were then cut across the green bean pods and examined with a 10-power hand lens. By this simple procedure it is possible to discover whether frozen green beans were frozen or thawed too slowly.

Basic investigations on the changing physical characteristics of vegetables during dehydration and rehydration are conducted in contract research at the University of California at Davis. Vegetables representing a range of dehydrating and rehydrating characteristics are under study. Differences in tissue structure were encountered in histological examination of onions, carrots, green beans, celery, green bell peppers, and mushrooms. The experimental approach has been to modify and refine laboratory techniques. Potassium permanganate was superior to other fixing agents for samples to be sectioned for electron microscopy. An epon resin was better than other embedding materials for sectioning samples. The amount of dehydration allowable without significant difference between texture of fresh and reconstituted vegetables varied widely between the vegetables. Enzyme activity in blanched or unblanched dehydrated vegetables was investigated; in unblanched dehydrated onions enzyme activity recommenced during rehydration, even of onions that had been dried well beyond the reconstitution texture-breaking point where irreversible changes must have occurred.

B. New and Improved Products and Processing Technology

1. Dehydrated Vegetables. Research to provide chemical and technological information was initiated by comparing a number of samples of commercially

produced air-dried, unblanched celery with an experimentally produced dried celery. The commercial samples had lost up to a quarter of their original chlorophyll content by conversion to olive-brown pheophytin but the laboratory sample has lost only about half that much chlorophyll. In the laboratory sample, most of the chlorophyll changed during the final drying stages. Higher air temperature in the final stages destroyed more chlorophyll. Gas chromatography determined the loss of volatile components during dehydration. Changes in chromatograms indicated increased formation of several highly volatile components as dehydration proceeded. However, over half of the original amount of several volatiles was lost during dehydration. Reference samples frozen in an air blast freezer also lost large amounts of volatiles. Dehydrated celery rehydrates very incompletely. After drying, cooked celery regains only between a third and a half of its original fresh weight. We have initiated studies to determine if altered processing conditions can improve rehydration.

Studies of foam-mat-dried tomato powder showed losses during long-term storage of lycopene, fructose, glucose, and amino acids to be greater at higher storage temperature. Lycopene is very rapidly destroyed by exposing the dry powder to air. An atmosphere containing less than 0.5% oxygen is needed to retain the red color. Sulfur dioxide treatment before drying stabilized glucose and fructose. Salt increased the loss of all four components. Calcium versenate improved stability somewhat. The most notable change was the disappearance of glutamic acid during warm storage. Interestingly, this amino acid is virtually absent from the more stable foam-mat-dried pineapple and grapefruit products.

2. Dry Bean Products. Development of quick-cooking Lima beans is supported in part by the Lima Bean Advisory Board which provides salaries for two scientists. Dry Lima beans were vacuum hydrated at room temperature in a solution of several salts and dried in low velocity air at moderate temperature and low relative humidity. This treatment reduced rupture of seed coats. Several combinations of salts in solution were tested to reduce cooking time. A combination of four mineral and organic salts was most satisfactory. Following treatment, the dry beans could be cooked in 35 minutes without prior rehydration. Without treatment, the same beans would require overnight soaking and 60 minutes or longer to cook to the same tenderness. The quick-cooking Lima beans have shelf lives equivalent to unprocessed dried Lima beans. Only one of the salts used would require Food and Drug Administration approval and it has already been accepted in other food applications. The treatment was applied successfully to prepare quick-cooking pinto and kidney beans, soybeans, and dry whole peas. Studies on composition differences between unprocessed and quick-cooking Lima beans seek basic information on mechanisms involved.

Drum-dried precooked powders from dried beans, peas, and lentils were developed on a laboratory scale. Scaling-up of such laboratory process to commercial scale depends upon operations, equipment, and cost data not available in the laboratory. Therefore, contract research was initiated at

Michigan State University to carry out pilot-plant development to determine what equipment should be used, how it should be operated, and what the cost of production will be for bean powders. Preliminary studies have been concerned with the adhesion of bean purees to the drying drum. Organic additives were tested, some of which doubled the output rate of the drum dryer. Improved methods for quality control in pilot-plant studies are being developed, including microscopic evaluation of prepared bean powders.

Transfer of energy by microwaves shows some promise for partial drying of cooked beans. Such beans must be air dired to final moisture. By this preparation "butterflying" (curling of the seed coats) is minimized and the product can be readied for serving in 10 minutes.

3. Frozen Vegetables. Basic research on processing frozen vegetables (see paragraph 8-A-6) yielded information on frozen green beans that may immediately change commercial practice. Conventional freezing of green beans on a belt or in a plate freezer damages the tissue by separating cells and rupturing cell walls. Very rapid freezing with liquid nitrogen prevents tissue damage and produces frozen beans that cook to a texture resembling fresh beans. Further studies indicated that total freezing in less than about 15 minutes damages the bean cells very little. Slower freezing causes moderate to extensive damage and results in products that have flaccid texture and sloughing surfaces when cooked. Freezing durations of less than 15 minutes can be achieved in the new fluidized bed freezers and possibly on modified belt freezers. It seems to us that material improvement in the texture of frozen green beans can be achieved with existing commercial equipment. Cooperative work during the next harvest season will test this idea.

4. Processing Quality of Vegetables. Studies on processing characteristics of vegetable varieties are conducted cooperatively with the Washington State Agricultural Experiment Station. Commercial production of asparagus in Washington is increasing and factors such as seasonal variation, cultural treatment, variety, length of green spear, diameter of spear, post-harvest storage, and enzymatic treatments on fiber are under investigation. Samples of canned and frozen asparagus were prepared for organoleptic evaluation and fiber analysis. Irrigation, cultural treatments, blanching, and harvest procedure changed the fiber little, but varietal differences were important in the first year of this study. Five varieties of green beans were evaluated after liquid nitrogen (see paragraph 8-B-3) freezing and conventional freezing techniques. All varieties were firmer when frozen with liquid nitrogen. No differences in firmness were found between midseason and late harvest beans.

In the manufacture of tomato products (puree, paste, catsup, etc.) consistency of the finished product is important and processing variables can materially affect consistency. Consistency is lost by enzyme action when raw tomatoes are broken or crushed before pressing. Heating at the time of crushing to destroy enzymes is common in the industry. Recent data indicate that increases in consistency and better control of consistency may be obtainable through inhibitors of the two enzymes pectin methyl esterase and polygalacturonase.

It is also important in controlling consistency to detect variations in the raw material that will change the product. We have been working cooperatively with a committee of the Canners League of California to develop laboratory methods to make such detections. A test of incoming tomatoes correlated well with finished tomato paste consistency in pilot-scale operation at the University of California, Davis, Department of Food Science and Technology. Further comparative runs will be made during the coming year in pilot-plants, and in limited commercial operations cooperatively with industry groups.

5. Microbiology of Frozen Vegetables. The development of extensive sanitation programs in a number of large food freezing plants is reflected in a general lowering of microbial populations in frozen foods. For several years education and cooperative research have been conducted in the Pacific Northwest to assist in improvement of sanitation in frozen food plants. During the past year, a Department bacteriologist continued his survey of commercial vegetable freezing lines for bacteriological cleanliness. Samples collected from processing equipment showed that coliform bacteria survive and grow on typical machinery used in commercial food processing. Such a finding casts further doubt on the common opinion that all coliforms indicate fecal contamination. It also makes imperative the elimination of slimes from food processing lines. High-level in-plant chlorination will eliminate slimes effectively. Laboratory studies are in progress on growth factors of Pseudomonas aeruginosa, a common contaminant of frozen vegetables. Increased carbon dioxide tension changed metabolism of the organism and the organic acids produced.

6. Removal of Radioactive Fallout. A research contract was initiated with the National Canners Association's Western Laboratory in Berkeley, California to investigate the extent to which external and internal strontium-90 from radioactive fallout can be removed from vegetables and fruits by modification of washing, blanching, and other unit operations of processing. Peas, potatoes, tomatoes, snap beans, spinach, sweet corn, broccoli, peaches, pears, apples, Meyer lemons, and strawberries are being studied. A growth chamber was constructed to keep birds from removing material and to collect radioactive runoff water. Preliminary experiments indicated that all crops in soil enriched to contain over 4,000 p.p.m. of strontium in the top four inches grew abnormally. At 830 p.p.m. of strontium peas, spinach, broccoli, and potatoes grew well. Such culture is necessary to provide material with controlled variable strontium contamination in order to study methods for its removal. Laboratory methods are being developed for the analysis of strontium and of radioactive strontium and cesium in vegetables and fruit.

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1/ Research supported by P.L. 480 funds.

AREA NO. 9. CASTOR, SAFFLOWER, AND OTHER WESTERN OILSEEDS--PROCESSING AND PRODUCTS

Problem. To provide valuable diversification crops for the acreage withdrawn from the production of cotton, wheat, feed grains, and other surplus crops, we must expand the markets for crops such as castor and safflower. Large amounts of safflower are exported and research is needed to insure the continuance and expansion of this promising market. Also, these crops are so new to our agricultural economy that their market potential has not been adequately developed. Castor and safflower have good potential because of the unusual properties of their oils. The possibility of large-scale increases in the production of these oilseeds would be strengthened if high-quality feed products could be developed from the oilseed meals. Basic information is needed on the composition of the oils and of the meals left after extraction of the oil, and this, in turn, requires the development of adequate analytical methodology. Rapid and accurate analytical methods are needed to control and improve the processing of the oils and meals for food, feed and industrial applications. Research on chemical conversion of the oils and evaluation of the modified products is needed to find new or improved large-volume uses. The high percentage of linoleic acid (essential fatty acid) in safflower oil points to a rapidly expanding use as a food oil. But this same fatty acid imparts a high susceptibility to autoxidation. Research is needed to stabilize safflower oil in various food products. Improved procedures for decorticating and processing castor and safflower seeds are needed. There is a particularly critical need to remove or destroy the allergenic and toxic components of castor meal which presently limit its use to fertilizer. Research to isolate and characterize the constituents in castor and safflower meals is needed to develop non-toxic, non-allergenic feed and food products of high value. Particular emphasis should be placed on developing safflower meal suitable for human consumption, opening an entry into the increasing edible protein export market. Basic and applied research is needed to prepare chemically modified products from the meals for industrial applications, to develop economical procedures for carrying out the modifications, and to evaluate the modified products.

USDA AND COOPERATIVE PROGRAM

In the Western Utilization Research and Development Division, both basic and applied research are conducted on castor and safflower seed at the Division headquarters at Albany, California, under contract in Arizona, and by P.L. 480 grant funds in India. Basic, compositional studies on castor seed meal are concerned with the resolution of its water-soluble proteins and determination of the nutrient properties for animal feed. Studies are conducted on the composition of castor and safflower oils and meals, and new analytical techniques are developed.

Applied research on castor meal has as its objective the development of economical methods for deallergenizing the meal without impairing its nutritive quality, to increase its value as an animal feed ingredient. Castor oil and its major constituent, ricinoleic acid, are being studied to provide for them new and improved industrial applications. Thus, methods are being developed for the preparation of various types of polyurethane foams incorporating castor oil or its derivatives. Procedures are also being devised for the preparation of chemical derivatives from ricinoleic acid, including ketones, acrylate esters, ω-hydroxy acids, and halogen- and phosphorus-containing glycerides. Several of the latter compounds may be useful for improving the flame-resistance of castor-based polyurethane foams of the type which may be used for building insulation. The utility of various polymerizable monomers, e.g., acrylate esters from castor oil for the production of synthetic polymers for use in rubbers, plastics, etc., is being investigated under contract. Research has been initiated on the composition of new and commercially promising safflower varieties. Detailed studies are underway to evaluate variation of fatty acid, amino acid, protein, fiber, etc. with the types of seed. Oils from new and established varieties are being studied for oxidative stability which is needed for large-scale food uses. The meals are being evaluated as protein sources in animal rations. Research under contract is underway on the types and amounts of natural antioxidants in the various safflower seed oils.

The Federal program of research in this area totals 9.6 professional man-years, including contract research equivalent to approximately 1.8 professional man-years per year. Of this total 5.6 are assigned to chemical composition and physical properties; and 4.0 to new and improved products and processing technology. In addition, two grants on applications of research are sponsored under P.L. 480.

PROGRAM OF STATE EXPERIMENT STATIONS

Castor and safflower are of interest due to the unusual properties of their oils and as possible replacement crops. State stations are investigating agronomic and harvesting problems. Utilization research is limited to nutritional and chemical evaluations of the castor plant being done in cooperation with USDA. Objectives include study of: the toxic and hemagglutinating protein, ricin; use of castor meal as a supplemental feed for large animals; the role of ricinine in metabolism and physiology of the plant; and isolation and identification of the compound(s) responsible for the foaming of aqueous extracts of castor beans.

There are approximately 0.1 professional man years devoted to this study.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Detection of Allergens. Research to develop methods for the qualitative and quantitative analysis of allergens from castor seeds was completed, and

attention directed toward adapting such methods to other problems, including detection and control of toxic metabolites of mold contaminants. In the course of the research, human allergies were successfully detected by application of patient serum to monkeys and other primates. The results correlate with clinical skin tests of the serum donors. The animal test method was also used to determine degree of deallergenation of castor bean pomace by novel processes. Applicability to other agricultural problems was demonstrated in the evaluation of 57 species of Euphorbiaceae being considered as potential replacement crops. These plants were examined for cross-reaction antigens to evaluate the risks of dust allergies similar to or cross-reacting with castor pomace allergy, risks that may be encountered if such plants are introduced for wide-scale agricultural development. Cross-reactions were observed. In addition, unequivocal tests confirmed earlier findings that chlorogenic acids from coffee or other sources are not allergenic and have no cross-reactions with castor allergy, as has been published elsewhere. Because chlorogenic acid occurs so widely in plants, the implications of its reported allergenicity could have had serious repercussions on market developments, particularly for fruits and vegetables.

2. Oilseed Components. Further compositional studies were conducted with 24 varieties of thin- and thick-hulled safflower seeds. Hull and fatty acid content were the main factors in seed variability. Hull content ranged from 50% to 18%, and oil content from 30% to 45%. Gas liquid chromatographic analyses indicated large differences in fatty acid content between varieties. Thin-hulled varieties contained about 80% linoleic acid and 12% oleic acid, whereas some thick-hulled varieties had as much as 83% oleic acid and only 10% linoleic acid. The difference in fatty acid content of oils attainable by varietal selection and development of safflower offers a great potential in developing specific oils for specific uses. Safflower oil for food use must be stabilized against undesirable polymerization at high temperature. Combinations of hydrogenation and antioxidant addition provided some protection, but basic investigations must continue.

Synthesis of acrylate esters of hydroxy acid derivatives resulted from various approaches utilizing acid- and base-catalyzed alcoholysis of acid chlorides and anhydrides, solvolysis of various alcohol derivatives, and direct esterification. A simple two-step process leading to good yields and high purity of methyl 12-acryloxystearate was developed and scaled up. The application of new methods for acrylate ester synthesis in high yields and purity will provide monomers for preparation of plastic coatings, lubricants, and other industrial products of high value. Several commercial dehydrogenation catalysts were evaluated for ketostearate preparation. The low cost of highly efficient dehydrogenation catalysts should make ketostearate an attractive industrial product.

Thin-hulled safflower varieties offer higher oil yields than thick-hulled seeds do, and the seed meal has higher feed value because of its smaller amount of indigestible hull. However, a very promising brownstripe, thin-hulled variety produces oil that has an off-odor and a brownish color from

hull pigment. Research is being conducted to eliminate the unpleasant odor and reduce the transfer of color to the oil. Large-scale decortication of safflower varieties produced kernel-free hull from which volatiles will be collected, concentrated, and analyzed by gas-liquid chromatography to provide leads for the study of off-color development in safflower oil. Good yields of high-protein, low-fiber flour were obtained from the thin-hulled safflower seeds. Contract research at the University of Arizona in Tucson was initiated to separate, purify, and characterize naturally occurring antioxidants in safflower oil. Plant breeders of the Arizona Agricultural Experiment Station are supplying new promising safflower varieties for evaluation. Information on oxidative stability of safflower oil from commercial and new varieties will be obtained.

B. New and Improved Products and Processing Technology

1. Product Developments. Rigid foams with good strength and insulating properties were prepared by a one-shot procedure using castor-based polyols directly with polymethylene polyphenylisocyanate. The procedure avoids preliminary time-consuming preparation of isocyanate-containing prepolymer, and it is less costly. With this procedure, castor-based polyurethanes reach a favorable competitive position relative to synthesized polyols from petroleum. We demonstrated that these foams can be made flame-resistant and non-burning by using brominated castor oil, and they are self-extinguishing when chlorinated castor oil is used. Preliminary work on phosphorus derivatives of castor oil has not yet yielded good non-burning foams.

At the University of Arizona, Tucson, contract research supported jointly by the Southern and Western Utilization Research Divisions is underway on polymerization and co-polymerization of selected monomers derived from oilseeds, preparation and characterization of new monomers, and preliminary evaluations of the polymers as industrial products. A large quantity of polyvinyl 12-hydroxystearate was prepared for use in polyurethane studies. The present contract is a continuation of joint support for the research at Tucson and will continue for five more years.

2. Animal Feeds. Primary consideration has been given to deallergenization of castor pomace to make castor a more valuable crop. Interrelationships of time, temperature, and lime concentration were determined in pilot studies. The higher the process temperature, the lower could be the process time and lime concentration necessary to effect acceptable deallergenization. Lime concentration of 8% with a one-hour process time at 120° C. was sufficient to obtain a satisfactory product. Lime treatment caused degradation and change in amino acid composition of castor meal. Lysine, arginine, threonine and serine were reduced, while glycine and alanine increased to a degree depending upon the severity of treatment. Arginine was partially converted to ornithine, and this conversion may afford a useful index of degree of deallergenization. If the index proves to be reliable in rigorous testing, it could substantially reduce the cost of analysis for production control in commercial deallergenization processes. A plant-scale trial of

lime-treating of castor meal was completed at the Baker Castor Oil Company's Los Angeles plant. Further testing on the plant-scale level must be done to complete the development of this process. Material has been prepared for feed evaluation. Additional pilot plant studies are in progress on ammonia and other basic treatments that show special promise.

Partially decorticated thin-hulled safflower seed produced good yields of high-protein (60%), low-fiber (3%) flour. When lysine and methionine were added to this meal, growth response was better in chicks than with any other protein source tested. When the protein level of the safflower-containing ration was reduced from 22% to 20% or 18% protein, growth rates decreased slightly, but results were still as good as those obtained with the higher protein positive control diets. Amino acid analyses on 25 different types of safflower seeds showed that lysine varied only about 10%. With the rapid increase in safflower production in the western United States, knowledge of food and feed values of the meal is essential if maximum value is to be obtained from this relatively new domestic crop.

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AREA NO. 10. SUGAR BEETS-- PROCESSING AND PRODUCTS

Problem. Sugar beets are mainly processed for sugar; a very small proportion is used for livestock feed. Sugar beets are declining in sugar content and rising in impurities. The traditional processing methods for sugar manufacture cannot cope efficiently with beets whose lower quality is due in part to excess nitrogen fertilizer, used to improve tonnage yields. Improved processing procedures should benefit both the growers and processors. It is known that small concentrations of certain chemicals in beets affect processing quality but not enough information is yet available to devise new economical procedures for high-impurity beets. Because costs of producing beets and processing sugar are rising whereas per capita consumption and price of sugar are essentially constant, all factors important to utilizing the crop must be examined to improve processing. There is still much to be learned about the composition of sugar beets, juices, pulp, and crude sugar. Sugar losses resulting from spoilage and respiration of beets held at processing plants cannot be prevented by existing methods.

USDA AND COOPERATIVE PROGRAM

Utilization Research on this Area has been discontinued. Research grants under P.L. 480 continue on basic research at Jerusalem, Israel and on product developments at Calcutta, India.

PROGRAM OF STATE EXPERIMENT STATIONS

The station program on sugar beet utilization is concerned with chemical composition of the beets. The effects of management, genetics, and environmental factors on yield and quality of sugar beets, including sucrose content of root and purity of juice is being determined. Other research, while serving projects designed to consider possibilities of growing beets in several new areas, also provides data on composition and the influence of environment and practices of fertilization and management on yield and quality of sugar beets.

The total research effort devoted to this work is about 1.6 professional man years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Sugar Beet Composition. Quantitative analyses for minor carbohydrate constituents of sugar beets in the presence of large amounts of sucrose have been quite difficult. Thin-layer chromatography is useful for qualitative but appears to have limitations for quantitative analyses. Paper chromatography promises to be suitable for analyses of both kestose and raffinose in beet juices. Gas chromatography of trimethyl silyl ethers of sugars has been a useful tool for quantitative analysis of beet sugars. Determination of raffinose using galactose oxidase was successful provided no other galactose-containing sugars such as melibose or galactinol were present in the system. No galactinol was found in sugar beets from some samples from California, but when galactinol is present in stored beets awaiting processing it causes errors in measurement of sucrose. A new supply of galactinol for analytical reference purposes was prepared by a new method from syrup supplied by the Great Western Sugar Company. Innovations in the galactinol preparation include fermentation of sugars with yeast, conversion of reducing sugars to acid by oxidation, ion exchange, and ion exclusion. Ion exclusion appears to be a new useful technique for separating classes of sugar and for separating sugars from each other. For example, nystose (tetrose), kestose (triose), sucrose, glucose and fructose have been separated in laboratory-scale ion exclusion columns.

Information on enzymes involved in sucrose degradation in sugar beet tissue is being obtained at the Hebrew University, Jerusalem, Israel under P.L. 480. A detailed quantitative survey of several important intermediates in the carbohydrate metabolism of sugar beet root was conducted. Results indicate that the process of sucrose degradation in beet root is highly controlled and invertase is not involved. Sucrose cleavage is catalyzed by the enzyme synthetase at a rate such that sugar phosphates do not accumulate. This means some step in the sequence of sucrose degradation is limiting, and that step may be the first cleavage of sucrose. Fifty other enzymes have been shown to be active in sugar beets. Invertase activity was at a very low level.

B. New and Improved Products and Processing Technology

1. Juice Recovery and Purification. The sugar beet industry is concerned about the declining sugar extraction record of the past 20 years. Factory production records between 1943 and 1962 indicate that decreased production may be the result of lower sugar content of beets, lower purity of extracted juice, and the extension of the processing season. In most factories, sugar production can be predicted by use of equations involving sugar content of beets and thin-juice purity. In other factories, the cause of sugar losses is unknown. Decrease in sugar yield was substantial over the 20-year period for processors whose seasons were materially extended, giving rise to deterioration of the stored beets. Raffinose, kestose, galactinol, and reducing sugars increase at the expense of sucrose during beet storage at the factory.

Studies have been conducted to find conditions for storing beets that prevent accumulation of these non-sucrose carbohydrates.

Preliminary pilot-plant trials of the pressing of lime-toughened sugar beet pulp were conducted by informal cooperation with both a sugar beet processor and with a press manufacturer. Modifications in the continuous vertical screw presses now used are necessary before commercial application can be evaluated unless trials can be made using the latest type of large-scale, twin-screw horizontal press.

Ion exclusion is a way of separating sucrose from molasses impurities by means of an exchange resin that is already bonded with charged molecules. The separation occurs because the sucrose invades the porous resin while the charged impurities are excluded. The virtue of this method is that no expensive and damaging chemical regeneration of the resin is needed. Studies of ion exclusion purification of sugar-salt mixtures and molasses were conducted using a 6-inch by 10-foot steam-jacketed resin bed. Many runs were made to define the range of operating conditions for such variables as temperatures, flow rate, load volume, and solids concentration. The results are being analyzed by means of a digital calculator.

2. Sucrochemicals. The domestic Federal program of research on sucrochemicals was terminated in response to recommendations of the Sugar Research and Advisory Committee in 1963. Prior to termination of research on sucrochemicals, negotiations had taken place to provide P.L. 480 grants for work in this area. One such grant, to develop modified sugars that could be used for the synthesis of useful plastic substances, is still in progress at Jadavpur University in Calcutta, India. Principal activity during the past year has been to find an optimum reaction condition for synthesizing amino sucrose via hydrazino sucrose starting from mesyl and tosyl derivatives. So far, mixtures of amino compounds of only very low yields have been obtained. In a study of the differences in reactivity of hydroxyl groups of alpha-methyl galactoside compared with those of alpha-methyl glucoside, methylation of galactoside gave a very small amount of crystalline product. Four types of sulphate esters were obtained by adding a calculated amount of chlorosulfonic acid to sucrose in pyridine. Larger amounts of these esters will be prepared for further study.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

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1/ Research supported by P.L. 480 funds.

AREA NO. 11. POULTRY-- PROCESSING AND PRODUCTS

Problem. The $1.6 billion poultry meat industry operates on very narrow profit margins. In order to keep abreast with developments in the modern food industry, poultry must be converted into a wide variety of products having high quality and improved convenience, at costs attractive to consumers and remunerative to the poultry grower. More information on the properties and processing of poultry is needed to enable us to better utilize poultry in a variety of forms attractive to consumers. Increased utilization of poultry would also serve toward eliminating our feed grain surplus, increasing returns to farmers and providing better products for American consumers.

Although poultry is an efficient converter of feed to meat, more grain is used by poultry per calorie of food produced than by any other commercial animal because a high percentage of the poultry diet is grain and because poultry meat contains exceedingly little fat. Furthermore, one-fourth of all grain fed to animals is used for poultry and egg production. Hence, increased consumption of poultry products would be an effective means of increasing markets for surplus grain. Also, the efficiency of feed utilization by poultry makes possible low prices within reach of more consumers. A still further benefit would arise from the increased use of poultry by improving the nutrition of consumers having diets now low in animal protein.

The consumption of poultry has steadily increased from a 1947-1949 average of 22 lbs. per capita to 39 lbs. for 1963. This important increase has involved price, quality of product, availability, and disposable income. Because of the current low profit margin it is impractical to increase consumption by lowering farm prices. Increased demand for and consumption of poultry will require higher quality and more convenient products and a greater variety to meet the desires of the modern consumer. However, in addition to greater returns from increased demand, a greater profit margin for the farmer can, of course, come from greater efficiencies in processing.

The trend toward convenience foods and further processing has primarily led to precooked poultry products which are generally less stable, more subject to warmed-over flavors, and more likely to provide texture problems than uncooked items. With the expansion of operation and the emphasis on continuous, more efficient processing, need has arisen for improved processing procedures for feather removal, chilling, tenderization, freezing, deboning, and commercial cooking. Lowering the cost and improving the quality of products that can be stored at ambient temperatures, such as canned, dried, cured, and irradiated products, offer potential for poultry utilization in domestic and export markets. As a foundation for applied studies, further knowledge is needed on the chemical nature of flavor and flavor changes in processing and storage, on tenderness development, and on proteins, lipids, and other components.

USDA AND COOPERATIVE PROGRAM

Basic and applied research on poultry meat and poultry meat products are conducted at the Division headquarters at Albany, California and by contract in Madison, Wisconsin, and Berkeley, California. Fundamental studies on poultry flavor are concerned with the identification of flavor precursor constituents in poultry meat and in the isolation and identification of volatile flavor components developed during the cooking of poultry. The chemistry of muscle protein and post-mortem chemical changes are investigated relative to the tenderness and other quality characteristics of poultry. The basic physiological character of feather release mechanism in fowls is studied to provide a foundation for improved feather removal. Applied research is conducted on the stability of cold-tolerant organisms; special problems of flavor, texture and stability of precooked frozen foods; and processing factors that influence tenderness of poultry meat.

The Federal program of research in this area totals 12.9 professional man-years, including contract research equivalent to approximately 1.7 professional man-years per year. Of this number, 4.3 are assigned to chemical composition and physical properties; 8.6 to new and improved food products and processing technology.

PROGRAM OF STATE EXPERIMENT STATIONS

State stations conduct both basic and applied researches on poultry and turkey meat and meat products. One phase of this work is directed to solution of problems associated with maintenance of the quality of fresh poultry. The effects of processing procedures such as feather removal, dressing, chilling and packing conditions are related to the bacteriological, organoleptic and physical properties of the finished product. Factors such as wholesomeness, microflora, condemnation losses and biochemical changes during processing must be evaluated.

There have been extensive new developments in work methods, equipment and facilities, and processing and packaging procedures. For example, continuous chillers have reduced the chilling time, increased uniformity and effectiveness of chilling and improved broiler quality. Current work involves observation of bacterial counts, water uptake, leaching of solids from carcasses and organoleptic quality as well as shelf-life of carcasses chilled by this technique.

Fundamental studies are concerned with the chemical and physical properties of poultry and turkey meat. Chemicals that affect the chemical bonding within and between protein molecules are used to study their effect upon the tenderness of the muscles after cooking. Results, to date, suggest that sulfur bonding as affected by chemical agents may be a factor in the ultimate tenderness of poultry meat. Studies which seek to determine the specific pathways and effects of post-mortem changes are continuing.

The influence of dietary fat upon the composition of the chicken carcass and the effects of increased polyunsaturated fatty acid content of the meat are being carefully evaluated. Special attention is being given the biological value of the protein and other constituents.

Continued research effort is directed to evolving an understanding of the precursors of chicken flavor and of the components which make up chicken flavor. Advanced techniques of flavor analysis are being used.

Basic microbiological studies continue to be directed to establishing the source of organisms, and the natural flora on poultry and poultry products; to the effects of organisms found in bruised or diseased tissue; to the build-up of organisms during the various stages of processing; and to means for extending shelf-life by retarding bacterial growth in poultry and poultry products. Another phase of the microbiological program deals with growth, survival and control of potentially pathogenic organisms.

New product research centers on accumulating fundamental information basic to the development of new or improved products. Significant results have been obtained in establishing the time-temperature relationships for processing turkey rolls. New approaches are sought to improve form, texture, flavor, juiciness and quality of products. Special attention is being given development of new poultry items from lower grades of poultry including ways to improve and utilize the carcasses of aged fowl.

Other investigations are concerned with insecticide residues in poultry and the effect of cooking and storage time and temperatures upon residue levels. In addition, market tests and economic feasibility studies seek to determine the market potential for new or improved poultry items.

A portion of the research on poultry products is conducted under the regional project NCM-40.

The research effort devoted to increased utilization of poultry and turkey products is approximately 19.2 professional man years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Post-Mortem Biochemistry and Tenderness. Basic investigations on the muscle chemistry of poultry are principally concerned with characterizing post-mortem changes in metabolites of the glycolytic cycle in relation to tenderization. An extremely labile metabolite, creatine phosphate, was previously shown to have a concentration dependent upon the conditions that prevail during slaughter. Electrically stunned birds had only one-eight as much creatine phosphate as did birds that were anesthetized with pentobarbital prior to slaughter. Studies were extended to include determination of adenosine triphosphate. This compound was significantly higher for birds

that had been electrically stunned. The time required for adenosine triphosphate to drop to 50% of its resting level was 7 hours for anesthetized birds and 3 hours for stunned birds. Since rigor mortis occurs when the muscle adenosine triphosphate level is reduced approximately 50%, these experiments show how anesthetization can significantly delay onset of rigor mortis.

Contract research at the American Foundation for Biological Research in Madison, Wisconsin was initiated on the histology of structural changes in poultry muscle. Electron microscopy of pre-rigor and post-rigor muscle revealed wide structural changes that can be induced by heating and by freezing. Rapid freezing and thawing cause some deformation of striations in pre-rigor muscle, but no substantial change has been observed in post-rigor muscle. Heating results in a great disturbance of the striations of pre-rigor muscle and disorganization of certain muscle structures, but very much less change in post-rigor muscle. To the extent possible, muscles were held in restraint during the treatments to prevent the drastic shortening that occurs upon thawing and to lesser degrees with other treatments. Myofilaments in muscles remained in their original longitudinal position if restrained during thawing, but they aggregated laterally to form dense, irregular bands. The combination of freeze-thaw-heat applied to pre-rigor muscle accentuated the large, irregular bands caused by thaw rigor. The aggregation of myofilaments quite possibly affects toughness of meat.

2. Chemistry of Poultry Flavor. We reported previously that hydrogen sulfide (H_2S) originates from the amino acids cystine and cysteins during cooking of chicken. The quantity of H_2S in freshly boiled, fried, or roasted chicken meat is sufficient to create an equilibrium H_2S partial pressure in air above the meat that is at least 4 to 34 times greater than the H_2S partial pressure at which the odor of pure H_2S in air can be recognized. Therefore, H_2S evidently contributes to the aroma of cooked chicken meat. However, the odor of H_2S cannot be recognized as a separate, distinct entity in this total aroma. Furthermore, the odor of H_2S over chicken broth was not recognized by an odor panel until enough H_2S had been added to it to cause an H_2S partial pressure of at least 36 microns, which is at least four times the level that is readily detectable in the absence of other odors. Evidently the characteristic odor of H_2S becomes masked or altered to a marked degree as it blends with other volatile components of cooked chicken.

The non-H_2S-sulfur content of chicken fat was increased by bubbling pure H_2S through 5% solutions of carbonyl compounds in chicken fat. These samples had potent new aromas. This indicates that cooked chicken aroma may also include aromas formed by reaction of H_2S with carbonyl compounds.

More than 200 components are evident in chicken meat volatiles chromatographed on a 300 ft. x 0.01 inch capillary column. By combining gas chromatography with mass spectrometry, 6 sulfur compounds, 5 aldehydes, 8 methyl ketones, 5 benzene derivatives, 3 furan derivatives, and heptane have been tentatively identified in this mixture. Obviously we confront a major task in determining

the identity, origin, and flavor significance of more than 200 volatile compounds. The intractable nature of this task indicates that we need to evaluate the significance of groups or fractions of the volatiles in an attempt to simplify the problem. Fractions that show no aroma contribution will not be chemically characterized in detail.

3. Physiology of Feather Release. Contract research on characterization of the physiological mechanisms that control feather tightening and release in poultry has been concluded at Michigan State University. The direct effect of the central nervous system on the force required to pull feathers was established. Changes in pressure measured within the empty feather follicles indicate that the constrictive force exerted on the feather shaft is by the follicle wall. Anesthetics, specific nerve-stimulating or nerve-blocking drugs, and mechanical or electrical stimulation produced parallel effects on intrafollicular pressure and feather-pulling force. Increase in intrafollicular pressure and feather-pulling force induced by injection of nerve-stimulating drugs into anesthetized birds was reduced or neutralized by the action of specific nerve-blocking drugs. Basic information thus obtained can serve as a foundation for development of methods for feather removal that are not dependent upon a completely empirical approach.

B. New and Improved Food Products and Processing Technology

1. Freeze-Drying. Product-in-tube and fin-tube rotating freeze dryers are being studied to establish operating conditions required for continuous freeze-drying of cooked chicken meat and other products. (In-put and outlet reservoirs allow for limited continuous drying without breaking the vacuum of the system.) Drying rates up to twice those of conventional tray and shelf freeze-drying were attained over a range of physical conditions in the new equipment. A short hexagonal tube dryer capable of handling small batches of product was built and is being used to determine the tumbling freeze-drying characteristics of several classes of products. General design features of a commercial-size unit are being developed. A method is also being developed for tumbling freeze-drying of liquid products. Liquids are formed into frozen pellets by letting drops fall into a chilled fluorocarbon refrigerant; the pellets then can be freeze-dried in a tumbling bed under vacuum.

A commercial evaluation was made by a cooperator showing a very clear advantage for our continuous unit over any freeze dryers ever made before. The specific advantages are rapid drying time, simple equipment, steady labor demand, and steady refrigeration load. To exploit the last advantage, we are examining several ideas for a continuous condenser.

Contract research at the University of California, Berkeley, was initiated to develop engineering information on rate-controlling factors in freeze-drying and methods for programming heat input and determining drying end-points. Equipment has been designed for the continuous weighing of pieces of poultry meat during freeze-drying and for determining surface areas and pore sizes in freeze-dried products. The contractor is also studying heat transfer to cold liquids that are in contact with water vapor which freezes into the

liquid. This will assist us in choosing among several of our ideas for continuous condensers.

Sensory evaluations of the texture and tenderness of frozen and freeze-dried poultry meats were compared with objective measurements of shear-resistance and water-holding capacity. Dark muscle rehydrated poorly and was tougher and drier than light muscle, yet dark muscle had a greater water-binding capacity than did light muscle after dehydration. Studies of protein extractability of dark and light meats are providing basic information that may help explain qualitative differences.

2. Tenderness of Poultry Meat. A small trained taste panel and a large untrained panel were used to compare subjective evaluations of toughness of turkey meat with objective shear-force measurements. The trained panel could distinguish differences of 4 pounds shear force. They commented that, in general, samples above 15 pounds shear force were tough. The larger untrained panel indicated toughness at a shear force above 20 pounds. Eight hours aging time was adequate to achieve tenderness in turkeys if the turkeys had been thawed before roasting. These studies remove any doubt that may exist concerning the necessity for aging turkeys prior to freezing; this costly procedure must continue until we can provide new information upon which to base improved processing methods. An untrained panel of about 100 people evaluated toughness differences in 9-week-old chickens. At least 4 hours aging was found to be desirable, even for birds that are thawed before cooking. Two types of shear measurement were used, which correlated well with each other and with the evaluations of the untrained panel.

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AREA NO. 12. EGGS-- PROCESSING AND PRODUCTS

Problem. The nearly $2 billion egg industry is periodically faced with burdensome surpluses that drive prices below the break-even point for many producers. The industry is also faced with declining per capita consumption. The estimate for 1965 is down 20% from the 1947-1949 average consumption. Because the demand for table eggs is inelastic, the increased utilization of eggs must come in the form of new egg products that compete by means of quality and convenience. Adequate knowledge is lacking of the properties, processing characteristics, and new product potentials of eggs to develop new markets. Present outlets for the 10% of egg production that is frozen or dried include the baking, confectionery, salad dressing, noodle, and baby food trades. Modified and new products emphasizing quality and convenience are needed to increase acceptance of egg products by these industries and to compete successfully with egg substitutes.

Increased utilization of eggs would not only benefit the producer, but would also diminish our feed grain surpluses since poultry and egg production account for about one-fourth of all grain fed to animals. Improved egg-containing products would benefit the producer in three ways: by providing an increasingly useful buffer for stabilizing egg prices; by providing additional uses and outlets for eggs; and by providing more remunerative outlets for wholesome eggs that are unsuitable for table use because of appearance or handling characteristics.

Egg processors have four general problems. First, the potential of yolk-containing solids in convenience foods can be fulfilled only with improvement of flavor stability, of dispersibility, and freedom from pathogenic Salmonella bacteria. Secondly, the processing costs of whites should be reduced and their utility improved. Third, further basic research on egg composition and components is essential to reach an understanding of physical and chemical changes induced by processing and storage and thus provide a rational basis for devising improved processes and products. Fourth, formulation studies designed to incorporate eggs into new household and institutional convenience products, are needed. This last study must encompass a full appraisal of physical, chemical, and microbiological problems peculiar to the formulated products.

USDA AND COOPERATIVE PROGRAM

In the Western Utilization Research and Development Division, a broad program of basic and applied research is conducted at the Division headquarters at Albany, California; by contract in Ames, Iowa, Ithaca, New York, and Davis, California; and by grant funds under P.L. 480 in France, Australia, and India. Fundamental research is conducted on egg proteins and their relations to the functional properties and quality of eggs, on egg lipids

and their role in off-flavor development in yolk solids, on the mechanism of bacterial penetration and survival in eggs, and on the bactericidal, antiseptic, anti-inflammatory, and food preservative properties of lysozymes and other components from eggs. Applied research is conducted on the stabilization of yolk-containing solids to increase the usefulness of eggs in dry mixes and other convenience foods, on new and improved drying procedures to make dried egg fractions and products more readily and more completely dispersible, on various methods of controlling Salmonella in eggs, and on factors in the handling of shell eggs that affect egg product quality and cost.

The Federal program of research in this area totals 13.9 professional man-years, including contracts and grants equivalent to approximately 3.4 professional man-years per year. Of this number 3.8 are assigned to chemical composition and physical properties, and 10.1 to new and improved food products and processing technology. In addition, three research grants on basic problems are supported by P.L. 480 funds.

PROGRAM OF STATE EXPERIMENT STATIONS

State stations maintain a continuing long-term program of basic and applied research on egg and egg product utilization. Its scope extends from concern for the quality of the freshly laid egg to research designed to maintain the quality and enhance the storage life of novel egg products.

The program of work concerned with maintenance of initial egg quality includes evaluation of: the influence of breed and strain of laying hen; the effects of diet; and variations in management and seasonal factors upon the physical and chemical properties of eggs and the relationship of egg constituents of the functional properties of the eggs. For example, the effects of different feed additives on yolk color and the subsequent quality of sponge cakes are being studied. The sponge cakes produced from light-colored and dark colored egg yolks are being compared by objective measurements of moisture, specific gravity, index of volume, compressibility and elasticity. Other research is concerned with determining the influence that shell characteristics, temperature, humidity, holding time in storage, washing, shell treatment and packing have upon egg quality and use. The relationship of shell thickness and breaking strength to retention of internal egg quality continues to receive special attention. The occurrence of pesticide residues in eggs and their elimination from eggs and egg products receives continuing study.

The frequent presence of salmonellae in feed and the implication of eggs as a source of salmonellae food poisoning is of great concern to the industry. Work which seeks to determine the source(s) of contamination of egg products is in progress. Data indicate that most Salmonella species occur not as egg interior contaminants but as egg shell contaminants. It would seem necessary, therefore, to develop methods for eliminating salmonellae at the processing plant in addition to elimination from the feeds and farms in general. Significant work is being carried out on egg product pasteurization efficiency

as affected by pH and other factors. This work has previously shown that Staphylococcus and Salmonella organisms have maximum heat resistance near pH 5.0. Related research has shown that the pH at which liquid whole egg is pasteurized and centrifuged affects its physical, chemical and functional properties. In other studies, the fluorescent antibody technique is being used to attempt to identify small numbers of Salmonella organisms in eggs.

Basic studies designed to more fully characterize egg composition, structure, biological activity and functional properties continue. A comprehensive investigation of the comparative biochemistry of the proteins of eggs is going forward. Investigations of the cholesterol and other constituents of eggs and factors affecting these constituents are continuing to provide useful information related to public health aspects.

Microbiological studies continue to include concern for bacterial spoilage of shell eggs, mode of entry of organisms into eggs, action of proteolytic and lipolytic enzymes of psychrophiles in deterioration of eggs and development of control methods. Other work relates to controlling bacterial contamination in new products.

Processing research includes evaluation of cleaning, washing and pasteurizing procedures. The effect of spray-drying egg white at various pH levels and the subsequent influence upon the resulting product continues to receive intensive study. Fractionation of egg proteins and other components has revealed that a number of changes occur due to freezing and spray-drying.

Product development work continues to seek new and novel egg products. Shell-less eggs, frozen eggs, frozen egg products and egg combination products receive some attention.

The costs, efficiency and economic feasibility of egg processing operations continue to be researched. These engineering economic studies are essential to evaluation of alternative methods of processing and utilization of eggs.

A portion of the work on egg utilization is carried out under the regional project NCM-40.

There are about 10.5 professional man years devoted to egg utilization research at the State experiment stations.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Egg Proteins. To facilitate the study of the nature of changes caused by processing and other treatments, work has been conducted on the development of feasible chromatographic methods of separating egg white proteins. Diethylaminoethyl cellulose (DEAE cellulose) has given the best resolution of the egg white proteins. At least 18 components can be detected by the procedure developed, but the fractions are not analytically pure.

Nevertheless, the separation patterns are sufficiently reproducible to show difference between the composition of individual eggs.

In studies of the effect of pasteurizing temperatures on egg white proteins it was observed that lysozyme is much less stable in egg white than it is when separated from egg white. Basic study of this phenomenon showed that it is the ovalbumin component of egg white that causes lysozyme to be less stable to heat in egg white than in buffer. The inactivation reaction occurs more rapidly at alkaline pH values and is quite rapid under conditions that do not denature either ovalbumin or lysozyme. Comparative studies with cysteine (an -SH compound) strongly indicate that the -SH groups of ovalbumin rapidly reduce the S-S bonds of lysozyme at the pH of egg white when the temperature is raised to 60° C or above. The possibility that the newly discovered reactivity of the -SH groups of ovalbumin influences the stability of egg white under milder temperature conditions is being investigated.

A study of the physical chemical properties of the unique egg white component, ovomucin has been initiated. Ovomucin is a complex mixture of mucoproteins and is reputed to play a major role in establishing the viscosity and the thick-white properties of egg white. With physical chemical methods now available it should be possible to define the basic properties of ovomucin and to relate these to the gross properties of egg white and egg products.

Basic investigations of lysozymes from eggs and other animal sources are continuing at the University of Paris, France, supported by P.L. 480 grant. Lysozymes are proteins with enzymic, bactericidal, and other biological properties. To throw light on the structure of the active site in the lysozyme molecule, purified lysozymes from hen eggs, human blood leucocytes, goose eggs, and turkey eggs are being compared as to substrate specificity of the enzymic activity. Enzymic action in the digestion of mycobacteria cell walls indicates that the most basic lysozyme from eggs reacts more rapidly than does the enzyme of human origin, which is slightly less alkaline.

Research supported by P.L. 480 funds at the Indian Institute of Science in Bangalore is concerned with changes in properties of egg yolk proteins resulting from freezing. The gelation of egg yolk after freezing and thawing is specifically under investigation. Protein-splitting enzymes such as chymotrypsin, trypsin, and papain, as well as collagenase and a crude phospholipase isolated from Russel's viper venom inhibited gelation, whereas carboxy peptidase had no effect. Another phospholipase from a bacterial source did not inhibit gelation although it increased the opacity of the yolk plasma. Basic peptides were released from egg yolk proteins by the proteolytic enzyme treatment. Amino acid analysis indicated that the released peptides contained lysine and arginine, probably released as terminal groups from the protein molecules.

Studies of the mechanism of transformation of ovalbumin into S-ovalbumin were initiated, with P.L. 480 funds, at the Commonwealth Scientific and Industrial Research Organization, Ryde, New South Wales, Australia.

S-ovalbumin is more heat-stable than ovalbumin from which it is formed. To elucidate their structural differences, two approaches are being used: one involves the use of a denaturing solvent, formamide, which causes the organized protein molecule to unfold or stretch out into a random-coil chain. If the difference between ovalbumin and S-ovalbumin involves only internal structure and a difference in the weak bonds that hold the chain in the folded state, the two proteins should be identical when completely unfolded. Ovalbumin and S-ovalbumin, however, are different when completely denatured with formamide. The difference is reduced by adding a reagent that breaks the chemical bond between sulfur atoms of two amino acids. Therefore, the difference appears to be related to the position of the disulfide cross-linking. The second approach is designed to test the hypothesis that the S-S bonds of the two proteins are in different positions. If this is the case, enzymic hydrolysis of the proteins should yield polypeptides of differing composition because the amino acids adjacent to the different S-S bonds would almost certainly be different. Only those fragments that might be involved in a disulfide cross-link are being examined.

B. New and Improved Products and Processing Technology

1. Bacterial Spoilage of Eggs and Egg Products. An improved method has been developed for pasteurizing egg white to provide positive control of Salmonella, even under high levels of contamination. Pasteurization temperature for untreated egg white is limited by the instability of the protein conalbumin, which coagulates below the kill temperature for Salmonella. Iron-conalbumin is known, from our fundamental protein studies, to be more heat-stable than is conalbumin. The iron in egg yolk contributes to conalbumin stability so that whole eggs can be pasteurized without undesirable protein coagulation. We found that aluminum salts also stabilize conalbumin against heating, and they are more desirable than iron is for treating food, because they do not cause a color change in the product. Thus, pasteurization temperatures for egg white can be increased to provide Salmonella kills of inoculations as high as 10^8 with only minor damage to the functional properties of the egg white. Pasteurization at 60^o to 62^o C. is possible without coagulation of protein and without loss of volume in cakes made from the pasteurized eggs. However, increased whipping time is required. Where the whipping time increase is undesirable it can be overcome by adding a whipping aid, such as triethyl citrate. Commercial-scale pasteurization trials, using the aliminum addition, were made in several large processing plants. Over 30 concerns have applied for licenses to operate under the patent application. Pasteurized, Salmonella-free egg white is available from a number of processors. Eventually wide commercial adoption of this pasteurization treatment seems inevitable because of the simplicity of the treatment and the rapidly growing concern for Salmonella poisoning in foods.

Contract research to determine factors contributing to Salmonella contamination is continuing at Iowa State University in Ames. Feeding viable Salmonella to laying hens did not produce contaminated eggs. In processing plants, studies of work interruption of short or long duration for equipment

cleanup indicated that total counts in broken-out eggs may build up if clean- is superficial. The use of chemicals to pasteurize eggs is being investigated under contract research at the University of California at Davis. Purified iron conalbumin, high-quality ovomucoid, and antibodies in the form of rabbit antisera to whole chicken egg and to crystalline conalbumin will be used to determine the effects of chemical pasteurizing agents on the chemical, enzymatic, and biochemical properties of egg white constituents. A research grant to Cornell University was made for studies on the selenium metabolism of Salmonella because these microorganisms grow more readily on selenite media. Relative growth of Salmonella and E. coli on low-sulfur media indicate that selenium stimulates Salmonella growth but inhibits E. coli. The selenium-to-sulfur ratio may be critical in the development of selective media for Salmonella isolation and analysis.

2. Egg Powders. Functionality and stability of egg powders as affected by the addition of carbohydrate and by several processing variables have been studied. Gas-injection spray-drying, tested on a commercial scale at two processing plants, improved flowability and dispersibility of the powders. Bulk densities were lower and particle size was larger than for spray-dried egg powder without gas injection. Whole egg and yolk solids of various formulations were also prepared by forced-air drying of mechanically preformed foams and agglomeration of conventional spray-dried powders. Addition of carbohydrate and acid to egg white stabilized the dehydrated product and avoided the usual deglucosing step. The new product is superior to untreated spray-dried white in flavor and functional properties and is comparable to spray-dried deglucosed egg white. The increase in adventitious bacterial contamination that takes place in the usual deglucosing process can be avoided because the egg white can be dried immediately after carbohydrate and acid are added.

3. Precooked Frozen Foods. The principles governing the reactions of ingredients of prepared foods subjected to frozen storage were investigated. An increase in the use of eggs in such products is being sought through studies of the stability on freezing of foods involving egg foams. Both whole-egg and egg-white dessert soufflés have been studied. Egg-yolk concentrations ranging from 9 to 13% and salt concentrations from 0 to 0.6% did not affect the volume of whole-egg soufflés. Sugar helps stabilize egg-white foam. Sugar concentration and its distribution between yolk and white portions of foams affect both the original volume and the storage stability. For products low in sugar, we have found commercial stabilizers that improve the frozen storage stability of soufflés. Methyl cellulose was superior to other commercial stabilizers tested.

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AREA NO. 13. PHARMACOLOGY

Problem. Advances in agricultural science and processing technology necessitate the use of an increasing number of new chemical compounds whose safety must be established. The mutual interests of both the producers and the consumers of agricultural products, demand that public agencies participate in securing unequivocal evidence of safety before products of advanced technology are marketed. This responsibility is particularly acute where a public agency, such as the Department of Agriculture, contributes to technological developments that result in intentional or unintentional addition of untested components into foods, feeds, or into materials contacting the persons of consumers, or developments that result in the introduction, concentration, or modification of natural components in a way that may have an adverse physiological effect on consumers. Types of materials that require continuing surveillance include food additives, inadvertent residues of pesticides and other useful agricultural chemicals, antibiotics and medicinals, and the naturally occurring chemical constituents of physiological importance. In the areas of interest to Agricultural Utilization Research and, in particular, in connection with process and product developments of the four Utilization Research Divisions, such compounds must be tested by short- and long-term ingestion in experimental animals, such as rats and dogs, to secure toxicological data required by the Federal Food and Drug Administration to establish safety and legal certification for their use. The unequivocal establishment of safety for any useful chemical involves much more than merely conducting animal feeding tests on a routine service basis. It often requires original chemical analytical procedures and metabolic fate studies in experimental animals, as well as new methodology and observational techniques, to study of new chemicals. Each assignment in this field is a new area for original, often fundamental research.

A new dimension has recently been added to the problems in pharmacology facing the Department of Agriculture by the recent discovery that the mold Aspergillus flavus growing on peanut meal produces toxic compounds which are carcinogenic. Since it is unlikely that production of toxic compounds is limited to Aspergillus flavus, the compounds produced by any mold on any agricultural product should be tested for acute and chronic toxicities, metabolic fate, and mechanism of producing physiological effects.

USDA AND COOPERATIVE PROGRAM

Pharmacological investigations supporting the Department's utilization research and development program are conducted in the Western Utilization Research and Development Division at Albany, California and by contract at Davis, California. Agricultural products, and additives required to preserve or otherwise treat them, are investigated as they may cause toxic or allergenic reactions. Laboratory methods for discovering the metabolic fate of chemical compounds in animal physiology are developed and applied to problems in the utilization of farm products.

Plant constituents that exert deleterious or beneficial effects on animal growth are studied to determine quantitative responses.

The Federal program of research in this area totals 13.0 professional man-years assigned to pharmacology investigations.

PROGRAM OF STATE EXPERIMENT STATIONS

As public agencies concerned with safety and wholesomeness of agricultural commodities, the State stations carry on a continuing research program on the pharmacological aspects of plant constituents.

Surveying the occurrence of biologically active substances in foods, feeds, fungi, weeds, and other plant products, pharmacology-oriented scientists devote attention to the presence of such toxic products as alkaloids, hemagglutinins, glycosides of cyanides and coumarins, and cumulative heavy metals (lead, mercury, copper, selenium, and molybdenum). Fractionation studies are conducted to determine the site of formation of toxic substances in a plant and their distribution and extent throughout seeds, roots, leaves, and stems. Work is underway to develop methods of detecting, purifying, and isolating biologically active agents. Dosage levels to determine chronic and acute toxicity are under investigation with the aid of bio-assay techniques and histologic examinations of the involved animal tissues.

Studies are also designed to investigate the biochemical processes by which these agents produce their physiological effects. Not only biosynthesis and translocation in plants but also metabolic pathways in animals are followed to understand their modes of action, detoxification, accumulation or elimination. Specific examples include studies dealing with coumarin in sweet clover, thyroid stimulants in grass silage, muscular dystrophy factors in Bermuda grass, plant estrogens, nucleic acid synthesis in avian tumors, and characterization of hemagglutinins in legume seeds.

Research is in progress on new chemical and biological methods for testing and evaluating non-nutritive additives in foods and feeds. The content of drugs, antibiotics, and pesticide residues in feeds can now be determined rapidly even when present at low levels using polarographic or gas chromatographic procedures. Studies on small aquatic animals, such as Daphnia, as toxicological test animals for economy and speed are offering promise. Enzymatic changes in the liver of rats after the ingestion of DDT or dieldrin are being investigated with respect to borderline nutritional levels of nicotinic acid and other B vitamins.

Much effort is being devoted to biologically active substances which are produced by growth of fungi in animal feeds. Work on concentrating, isolating, and identifying the mycotoxins is proceeding along with corollary investigations on determining their mode of action and methods of assay.

The total number of professional man-years devoted to pharmacology research by the State stations is approximately 16.1.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

1. Citrus Products. The metabolism of caffeic acid depends upon microorganisms in the gut rather than upon endogenous reactions within the animal body. This observation led us to initiate studies of metabolism in vitro that may lead to a more basic understanding of the metabolic fate in animals of minor phenolic constituents of citrus. Freshly voided animal feces were incubated with various substrates, such as the flavonoids and phenolic acids common in citrus fruits and other plant materials. Preliminary indications of the nature of the primary metabolic breakdown of quercetin were obtained. Identification of several biofermentative phenolics produced from flavonoid nuclei is in progress. Other common phenolic compounds such as dihydroxyphenylalanine, xanthurenic acid, D-catechin, phloroglucinol, and rhamnetin are now being investigated by this procedure.

2. Fermentation Product from Starch. Polysaccharide B-1459 (xanthan gum), a fermentation product of Xanthomonas campestris, imparts high viscosity, even in low concentrations, to water and aqueous solutions, and it does not thin out when heated. Its potential as a food additive depends upon its toxicological safety, which we are evaluating. When the whole dried fermentation medium containing polysaccharide B-1459 was fed to rats at levels up to 10% of the diet for 90 days, no unfavorable effects were encountered. We conclude, therefore, that no toxic metabolites are produced during biosynthesis of the substance. Our safety evaluation data, obtained with rats and dogs, will be included in any petitions to Food and Drug Administration pertaining to a proposed use of the polysaccharide as a food thickening agent.

3. Toxic Fescue. Attempts made over several years to isolate toxic substance from fescue forage have failed. Now we have located, in fungi isolated from toxic fescue samples, the apparent source of the toxicity. Pure cultures of the toxic strains of fungi were grown on nutrient agar and on aqueous substrates. The agar cultures were extracted with ether and transferred to olive oil for rabbit skin toxicity tests, and the particulate-free filtrates of the aqueous media were injected intraperitoneally in mice. Rabbit skin reactions correlated well with the toxicity observed in mice. Death of the mice was due to massive internal hemorrhage. For further testing in cattle, large-scale production of the toxigenic strains of fungi is in progress. Mold-inoculated hay will also be fed to chickens to see if vesicular dermatitis results. If this test is positive, it may provide a method for screening toxic fescue hays.

4. Mycotoxins. Serious attention is being focused on mycotoxins. Aflatoxin has been demonstrated to occur in oilseeds and has caused hepatomas in trout and ducklings. Swine and cattle are known to be susceptible to moldy corn toxicoses. Approximately 10,000 human casualties in Russia between 1943 and 1951 were attributed to toxic effects of molds present on over-wintered wheat, barley, and millet. Russian scientists have demonstrated that at least 24 strains of 12 different genera of molds found on these grains can produce substances toxic to humans.

Several procedures are available for the detection and estimation of aflatoxin in agricultural products. Once aflatoxin is detected by thin-layer chromatography of extracts from suspect material, its presence is verified by bioassay, by crop-tubing extracts to ducklings, or by injection into chick or duck embryos. Duckling feeding tests of suspect agricultural products without the preliminary extraction are also useful; mortality, growth retardation, and liver histopathology correlate with the chemical estimate of aflatoxin.

Chemical modification of aflatoxin was investigated. When crystalline aflatoxin B_1 forms addition products with hydroxylated solvents such as formic or acetic acids in the presence of a strong acid catalyst, loss of biological activity results. Metabolic fate studies were initiated. Rats fed radioactively tagged aflatoxin B_1 excreted much of the aflatoxin in the urine and feces. Small amounts, however, are detectible in tissue extracts two days after the feeding. A large-scale swine-feeding test, involving peanut meal at graded levels of aflatoxin up to 1500 parts per billion, has been conducted by contract by the University of California at Davis; evaluations are in progress. The peanut meal for this test was prepared by the Southern Utilization Research and Development Division using naturally contaminated meals and meal that was fortified with isolated aflatoxin to obtain the highest level. A subsequent test at Davis is in progress with peanut meal prepared by the same Division using aflatoxin isolated by the Northern Division.

5. Toxicity of Potential Replacement Crops. The toxicity of Cassia hirsuta and Astragalus hamosus (new sources of polysaccharide gums) was investigated by feeding the seeds to rats. When the Cassia seed was fed at a dietary level of 5%, mortality was 100%, but no deaths occurred and growth was normal when the Astragalus material was fed for 4 weeks at a dietary level of 10%. When Cassia was fed at the 1% level, all rats survived, but growth was inhibited. The toxic factor in Cassia appears to be heat-labile. No rats died and growth was normal in rats fed for 18 days on Cassia seeds that had been autoclaved 1 hour at 15 pounds pressure. Tissues are undergoing histopathological examination.

6. Carbamate Toxicology. The potential hazard of carbamate cotton finishes is being investigated by means of rat-feeding experiments. Dimethylated alkyl carbamates, such as those used to treat cotton fabric, are being fed at the 0.1% level of the diet. No differences in growth, food consumption, or general appearance have been noted in rats fed methyl carbamate, ethyl carbamate, ethyl carbamate plus formaldehyde, or formaldehyde for 6 months.

7. Content and Availability of Fluorine in Chicken Bones. The poultry industry produces approximately one billion pounds of chicken backs and necks annually. These products are of low market value, and their use in homogenized form for gravy, soup mixes, and other formulations has been suggested. Feeding tests with weanling rats were conducted to determine the availability of fluorine from chicken-bone meal. Three levels of fluorine were added to the diet: 14, 28, and 56 parts per million. The onset of bleaching and the

degree of bleaching of rat incisor teeth was the same whether sodium fluoride or chicken bones containing 1070 parts per million of fluoride was the source of the fluorine. After rats had been on a diet containing 74 parts per million of fluorine (56 plus 18 from basal diet) for a year, the bones of rats fed sodium fluoride contained 3000 parts per million and those of rats fed chicken bone meal contained 2860. Bones of rats on the basal diet alone contained 645 p.p.m. These data and the observations on the incisor teeth demonstrate the equal biological availability of fluorine in sodium fluoride and in chicken bones.

8. Detection of Allergens. Research to develop methods for the qualitative and quantitative analysis of allergens from castor seeds was completed, and attention directed toward adapting such methods to other problems, including detection and control of toxic metabolites of mold contaminants. In the course of the research, human allergies were successfully detected by application of patient serum to monkeys and other primates. The results correlate with clinical skin tests of the serum donors. The animal test method was also used to determine degree of deallergenation of castor bean pomace by novel processes. Applicability to other agricultural problems was demonstrated in the evaluation of 57 species of Euphorbiaceae being considered as potential replacement crops. These plants were examined for cross-reaction antigens to evaluate the risks of dust allergies similar to or cross-reacting with castor pomace allergy, risks that may be encountered if such plants are introduced for widescale agricultural development. Cross-reactions were observed. In addition, unequivocal tests confirmed earlier findings that chlorogenic acids from coffee or other sources are not allergenic and have no cross-reactions with castor allergy, as has been published elsewhere. Because chlorogenic acid occurs so widely in plants, the implications of its reported allergenicity could have had serious repercussions on market developments, particularly for fruits and vegetables.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Pharmacology Investigations

Booth, A. N., Robbins, D. J., Ribelin, W. E., DeEds, F., Smith, A. K., and Rackis, J. J. 1964. Prolonged pancreatic hypertrophy and reversibility in rats fed raw soybean meal. Proc. Soc. Expt. Biol. and Med. 116(4), pp. 1067-1069.

Layton, Laurence L., and Greene, Frank C. 1964. Systemic allergic shock induced in monkeys passively sensitized by intravenous injection of human allergy sera. Internatl. Arch. Allergy and Appl. Immunol. 25, pp. 193-199.

Layton, L. L., Greene, F. C., Corse, J. W., and Panzani, R. 1964. Pure chlorogenic acid not allergenic in atopy to green coffee: A specific protein probably is involved. Nature 203(4941), pp. 188-189.

Layton, Laurence L., Greene, Frank C., and Panzani, Raphael; with assistance of Joseph W. Corse. 1965. Allergy to green coffee. Failure of patients allergic to green coffee to react to chlorogenic acid, roasted coffee, or orange. J. Allergy 36(1), pp. 84-91.

Layton, L. L., Yamanaka, E., Greene, F. C., and Perlman, F. 1964. Atopic reagins to penicillin, pollens and seeds. Dermatology Digest 3, pp. 86. (Abstract of paper which appeared in Internatl. Arch. Allergy & Appl. Immunol. 23, pp. 87-94, 1963.)

Masri, M. S., Hendrickson, A. P., Cox, A. J., Jr., and DeEds, F. 1964. Subacute toxicity of two chrysanthemumic acid esters: Barthrin and dimethrin. J. Toxicol. and Appl. Pharm. 6(6), pp. 716-725.

Masri, M. S., Jones, F. T., Lundin, R. E., Bailey, G. F., and DeEds, F. 1964. Metabolic fate of two chrysanthemumic acid esters, bathrin and dimethrin. J. Toxicol. & Appl. Pharm. 6(6), pp. 711-715.

Masri, M. S., Robbins, D. J., Emerson, O. H., and DeEds, F. 1964. Selective para- or meta-O-methylation with catechol O-methyl transferase from rat liver. Nature 202(4935), pp. 878-879.

Robbins, Dorothy J., Moulton, J. E., and Booth, A. H. 1964. Subacute toxicity study of a microbial polysaccharide fed to dogs. Food and Cosmetic Toxicol. 2(5), pp. 545-550.

VanEtten, C. H., Daxenbichler, M. E., Peters, J. E., Wolff, I. A., and Booth, A. N. 1965. Seed meal detoxication. Seed meal from crambe abyssinica. J. Agr. Food Chem. 13(1), pp. 24-27.

AREA NO. 14. REPLACEMENT CROPS-- UTILIZATION POTENTIAL

Problem. Farmers could achieve more economic use of their land if new and profitable crops were available that would have new end-use patterns. For example, it would be advantageous to develop a new oilseed crop yielding fatty acids that could find industrial use in applications for which acids from presently available domestic oilseed crops are unsuitable. To develop a new crop, three basic steps are involved: (1) survey of wild plants, in cooperation with plant scientists, to identify those having both potentially valuable components and promising agronomic potential; (2) detailed physical and chemical characterization of components of interest to obtain clues to likely end uses; (3) selection of the most promising species, followed by additional research to explore uses and demonstrate industrial potential, and by additional agronomic research to establish proper cultural practices and select the best strains and varieties. Only after these steps have been successfully accomplished can a proposed new crop be offered to agriculture and industry for introduction and development. Obviously, a program of this type is a long-range one. Yet, whether the future of agriculture involves conditions of surplus, of greater emphasis on foods and feeds, or of pressure for greater national self-sufficiency, the nation will benefit from availability of practical crop plants to serve its needs.

To achieve the objective, survey and characterization work needs to be greatly increased, since the greater the number of species examined, the greater will be the opportunities for finding plants meeting the criteria of high utilization and agronomic potential. Work of the Department has already revealed several promising sources of new potentially valuable oils containing unique fatty acids such as hydroxy-unsaturated acids, capric acid, epoxidized acids, and unusual long-chain fatty acids. In order to demonstrate the potential of these new materials, further work is required on their physical and chemical properties and reactions, on processing to obtain maximum recovery from source plants, and on by-products from processing, such as oilseed meals.

USDA AND COOPERATIVE PROGRAM

Utilization research in this area has been discontinued at Albany, California. Contract research at Fargo, North Dakota equivalent to about 0.4 professional man-years per year will be continued.

PROGRAM OF STATE EXPERIMENT STATIONS

Discovery and preservation of valuable plant germ plasm is a continuing objective of the station program in new crops. Much of the research in this area is being done via four regional projects and in cooperation with regional centers. A large portion of the work is cooperative with USDA. Each year

many plant introductions are grown and evaluated. Annual and perennial crops possessing potential for industrial or agricultural use are further evaluated for agronomic and chemical qualities. These include crops for paper pulp, pigments, drugs, tannins, essential oils, insecticides, polysaccharide gums, and oils rich in acids of unusual structure. Assay of native and introduced tropical plants for products of economic value receives special attention. New varieties of fruits, vegetables, and grasses better resistant to disease and drought are continually sought.

Basic aspects of this program involve study of the biochemical and physiological basis for differences in crop plants. Attempts are made to determine if differences in biochemical or physiological processes can be associated with particular factors related to quality. Information concerning carbohydrate transformations is sought through study of carbohydrate formation and enzyme mechanisms. Horticultural speciality crops are gaining in importance. A number of studies are underway to facilitate rapid development of this industry.

The total scientific effort devoted to replacement crops is 8.4 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Dimorphotheca and Lesquerella Seed Oils. Seed oils of Dimorphotheca and Lesquerella species and their derivatives are under continuing analytical investigation. Correlation data between far ultraviolet absorption and structure of fatty acid esters were obtained for 32 compounds to provide useful information about fatty acid derivatives. Substantial quantities of seed oils of Lesquerella fendleri and L. gordonii were hydrolyzed and analyzed for hydroxy acid contents. Methyl ethers of hydroxy acid derivatives were prepared and evaluated by optical rotatory dispersion. Optical rotatory dispersion studies provide a rapid method for assigning absolute configurations to hydroxy acids. To date 14 derivatives have been studied which support earlier conclusions concerning the absolute configuration of 9-hydroxystearate. Absolute configurations of 14-hydroxyeicosanoate from lesquerolic acid and 12-hydroxystearate from densipolic acid were also determined. Low temperature alkaline cleavage techniques were developed to obtain improved yields of short-chain hydroxy acids to use as starting materials for film and fiber polymers. Keto esters were prepared from dimorphecolic and lesquerolic acids at low catalyst concentrations. The effect of hydroxyl group position on reactivity was noted.

B. Industrial Utilization

1. Industrial Products from Hydroxy-Unsaturated Oils. Contract research at North Dakota State University is concerned with fundamental information on drying properties of dehydrated hydroxy-unsaturated oils and their relation-

ship to quality in specialty coatings. Seven Dimorphotheca and one safflower isocyanate films were tested for drying properties, hardness, adhesion, and scratch resistance. Long-term weathering and fire cabinet tests show that Dimorphotheca oil in suitable combination with isocyanates and polyols, yield coatings with excellent to superior properties. The good properties of some of these new experimental products and the added fundamental information from the tests should help stimulate industrial interest in Dimorphotheca oil if varieties can be selected and cultural and harvest practices advanced to bring about a reliable commercial supply. Castor oil dehydrated with sulfuric acid catalyst was utilized as a model for evaluation of changes occurring during the drying of dehydrated Lesquerella oil films. Fundamental information on drying properties of dehydrated hydroxy-unsaturated oils has been obtained, including attenuated total reflectance and direct transmittance infrared spectrophotometric values and peroxide values by which chemical changes associated with after-tack of dehydrated oil films can be elucidated.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Chemical Composition and Physical Properties

McFadden, W. H., Day, E. A., and Diamond, M. J. 1965. Correlations and anomalies in mass spectra. Lactones. Analyt. Chem. 37(1), pp. 89-92.

Industrial Utilization

Knowles, R. E., Taylor, K. W., Kohler, G. O., and Goldblatt, L. A. 1964. Industrial oils from seeds. Hydroxy-unsaturated oils and meal from dimorphotheca and lesquerella seed. J. Agr. and Food Chem. 12(5), pp. 390-392.

Line Project Check List -- Reporting Year July 1, 1964 to June 30, 1965

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress (Yes-No)	Area and Subheading
W1 2-24 (Rev.)[1]	Air classified flours	Albany, Calif.	Yes	1-B-4
W1 2-27 (Rev.)	Wheat feed products	Albany, Calif.	Yes	1-C-1
W1 2-29 (Rev.)	Bread flavors	Albany, Calif.	Yes	1-A-6
W1 2-41	Wheat endosperm constituents	Albany, Calif.	Yes	1-A-1 1-A-3
W1 2-43 (Rev.)	Gluten foods	Albany, Calif.	Yes	1-B-2
W1 2-44	Protein interactions	Albany, Calif.	Yes	1-A-3
W1 2-45 (C)[1]	Water-dispersible protein preparations	Lafayette, Indiana	Yes	1-B-2
W1 2-46 (C)[1]	Chemical basis for cohesiveness in gluten	Kansas City, Missouri	Yes	1-A-1
W1 2-47 (C)	Elimination of microbial contaminants of wheat flour	Chicago, Ill.	Yes	1-B-7
W1 2-48 (C)	Identification of wheat proteins by radiotracer techniques	Pullman, Wash.	Yes	1-A-3
W1 2-49 (C)	Protein and lipid composition of spring and winter wheat	Manhattan, Kansas	Yes	1-A-5
W1 2-50	Mechanism of flour maturation	Albany, Calif.	Yes	1-A-3 1-A-4 1-A-5
W1 2-51	Compositional factors of wheat relative to continuous-mix processes	Albany, Calif.	Yes	1-A-1 1-A-3 1-A-4
W1 2-52 (C)	Wheat bran and aleurone pigments	Corvallis, Oregon	Yes	1-A-7
W1 2-53	Protein-rich fractions from mill run	Albany, Calif.	Yes	1-B-2
W1 2-54	Light-colored bulgur for specific markets	Albany, Calif.	Yes	1-B-1 1-B-6
W1 2-55 (Gr.)	Oxidation-reduction enzymes	Madison, Wisconsin	Yes	1-A-4
W1 2-56 (C)	Rheological study of doughs	Menlo Park, Calif.	Yes	1-A-2
W1 2-57 (C)	Carrying capacity of HRW wheats	Manhattan, Kansas	Yes	1-B-5
W1 2-58 (C)	Protein changes during malting	Minneapolis, Minnesota	Yes	1-B-1
W1 2-63	Rice product developments	Albany, Calif.	Yes	2-A-1 2-B-1
W1 3-16 (Rev.2)	Improved forage feed products	Albany, Calif.	Yes	3-B-2
W1 3-18	Phenolic components of forages	Albany, Calif.	Yes	3-A-1 3-B-1
W1 3-19 (C)	Autoxidation of alfalfa lipids	Berkeley, Calif.	Yes	3-A-2
W1 3-20 (C)	Products from southeastern grasses	Tifton, Georgia	Yes	3-B-3
W1 3-21 (C)	Alfalfa products	Lincoln, Nebr.	No[2]	
W2 2-7 (Rev.2)[1]	Molecular properties of wool and mohair proteins	Albany, Calif.	Yes	4-A-1

[1] Project discontinued during the reporting period.

[2] Recently initiated project.

Line Project Check List -- Reporting Year July 1, 1964 to June 30, 1965

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress (Yes-No)	Area and Subheading
W2 2-22 (Rev.)	Chemical treatment of wool for shrink resistance and other "easy-care" properties	Albany, Calif.	Yes	4-A-1 4-B-1 4-B-2 4-B-4
W2 2-24 (Rev.)	Effect of fabric construction and functional properties	Albany, Calif.	Yes	4-B-3
W2 2-28 (Rev.)	Mechanical behavior of wool fibers and fibrous assemblages	Albany, Calif.	Yes	4-A-2
W2 2-29 (Rev.)	Effects of radiation on natural and modified wools	Albany, Calif.	Yes	4-A-3
W2 2-32	New types of yarns and fabrics from coarse wools	Albany, Calif.	Yes	4-B-2
W2 2-34 (C)[1]	Chemical modification of wool to increase drying rate	Durham, North Carolina	Yes	4-B-4
W2 2-35 (C)	Wear-wrinkling performance of light weight wool fabrics	Washington, D.C.	Yes	4-B-3
W2 2-36	WURLAN treatment of wool top	Albany, Calif.	Yes	4-B-1
W2 2-37 (C)	High luster wool fabrics	Washington, D.C.	Yes	4-B-4
W2 2-38 (C)	High energy radiation of wool	Durham, North Carolina	No[2]	
W3 1-83 (Rev.)[1]	Flavonoids in citrus	Pasadena, Calif.	Yes	5-A-2 5-A-5 5-B-1
W3 1-88 (Rev.)[1]	Citrus essential oils	Pasadena, Calif.	Yes	5-A-1 5-B-1
W3 1-101 (Rev.)[1]	Fruit juice products and processes	Albany, Calif.	Yes	6-B-6
W3 1-112 (Rev.)	Dried fruit products and processes	Albany, Calif.	Yes	6-A-2 6-B-1
W3 1-117 (Rev.)	Fruit pigments	Albany, Calif.	Yes	6-A-1
W3 1-119[1]	Fruit flavor components	Albany, Calif.	Yes	5-A-3 5-B-3 6-A-4
W3 1-120 (C)[1]	Macadamia nuts products and processes	Honolulu, Hawaii	Yes	6-B-7
W3 1-121	Heat transfer surface fouling	Albany, Calif.	Yes	6-B-6
W3 1-122[1]	Texture of fruits and fruit products	Albany, Calif.	Yes	6-A-3 6-B-3
W3 1-123 (C)	Fruit leucoanthocyanins	Los Angeles, Calif.	Yes	6-A-1
W3 1-124 (Gr.)	Cell wall organization of fruits	Cambridge, Mass.	Yes	6-A-3
W3 1-125	Composition of desert grapefruit	Pasadena, Calif.	Yes	5-A-1
W3 1-126	Viniferous grape products	Albany, Calif.	Yes	6-B-4
W3 1-127	New fruit dehydration methods	Albany, Calif.	Yes	6-B-2
W3 1-128	Rancidity control in walnuts	Pasadena, Calif.	Yes	6-B-7
W3 1-129 (C)	Grape juice extraction	Davis, Calif.	Yes	6-B-4
W3 1-130	Date composition and products	Pasadena, Calif.	Yes	5-A-4
W3 1-131	Tropical fruit products	Honolulu, Hawaii	Yes	5-B-2
W3 1-132	Processing quality--Northwest fruits	Prosser and Puyallup, Wash.	Yes	6-B-5
W3 1-133 (C)	Products from desert grapefruit	Tucson, Ariz.	Yes	5-B-1
W3 1-134 (C)	Phenolics in canned apple juice	Fort Collins, Colorado	Yes	6-A-1

1 Project discontinued during the reporting period.

2 Recently initiated project.

Line Project Check List -- Reporting Year July 1, 1964 to June 30, 1965

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress (Yes-No)	Area and Subheading
W3 1-135	Grape product developments	Albany, Calif.	Yes	6-B-4
W3 1-139	Composition of lemons and lemon products	Pasadena, Calif.	Yes	5-A-1 5-B-1
W3 1-140 (C)	Improvement of canned ripe olives	Berkeley, Calif.	No[2]	
W3 1-141 (C)	Determination of bitter components in navel orange products	So. Pasadena, Calif.	Yes	5-A-2
W3 1-142 (C)	Improved fining methods for wine	Geneva, N. Y.	Yes	6-B-4
W3 4-74 (Rev.)[1]	Composition of dry beans re processing factors and product quality	Pasadena and Albany, Calif.	Yes	8-A-3 8-B-2
W3 4-77 (Rev.)[1]	Tomato concentrate and powder	Albany, Calif.	Yes	8-B-1
W3 4-79 (Rev.)	Effects of processing on potato product flavor	Albany, Calif.	Yes	7-A-1 7-B-1
W3 4-80	Effects of processing operations upon texture of frozen vegetables	Albany, Calif.	Yes	8-A-6 8-B-3
W3 4-81 (C)	Dry bean characteristics	Urbana, Ill.	Yes	8-A-3
W3 4-82[1]	Microbiology of frozen vegetables	Puyallup, Wash.	Yes	8-B-5
W3 4-83	Mechanism of anhydration in bacterial spores	Albany, Calif.	Yes	8-A-2
W3 4-84 (C)	Histological studies of vegetables for dehydration	Davis, Calif.	Yes	8-A-6
W3 4-85	Flavor of tomato products	Albany, Calif.	Yes	8-A-1
W3 4-86	Chemistry and enzymology of vegetable flavors	Albany, Calif.	Yes	8-A-1
W3 4-87 (C)	Removal of radioactive fallout	Berkeley, Calif.	Yes	8-B-6
W3 4-88	Processing quality--Northwest vegetables	Prosser and Puyallup, Wash.	Yes	8-B-3 8-B-4
W3 4-89 (C)	Dry bean and pea powder	East Lansing, Michigan	Yes	8-B-2
W3 4-90 (Gr.)	Sporulation of food spoilage bacteria	Urbana, Ill.	Yes	8-A-2
W3 4-91	Effects of processing variables on dehydrated vegetables	Albany, Calif.	Yes	8-A-5 8-B-4
W3 4-93 (Gr.)	Lipids in plant tissue	Davis, Calif.	No[2]	
W4 3-1 (Rev.)	Chemical derivatives of ricinoleic acid	Albany, Calif.	Yes	9-A-2
W4 3-2 (Rev.)	Foamed polyurethanes from castor oil	Albany, Calif.	Yes	9-B-1
W4 3-3 (Rev.)[1]	Pharmacology of castor bean allergens	Albany, Calif.	Yes	9-A-1
W4 3-5 (C)[1]	Polymerization of castor oil-derived monomers	Tucson, Arizona	Yes	9-B-1
W4 3-8	Castor pomace deallergenation	Albany, Calif.	Yes	9-B-2
W4 3-9	Safflower oil and meal	Albany, Calif.	Yes	9-A-2 9-B-2
W4 3-10 (C)	Antioxidants of safflower oil	Tucson, Arizona	Yes	9-A-2
W4 3-11 (C)	Polymerization of vegetable oil-derived monomers	Tucson, Arizona	Yes	9-B-1
W5 1-73	Biochemical studies of non-sucrose carbohydrates in sugar beets	Albany, Calif.	Yes	10-A-1
W5 1-75	Effects of non-sugar chemicals on processing	Albany, Calif.	Yes	10-B-1

1 Project discontinued during the reporting period.

2 Recently initiated project.

Line Project Check List -- Reporting Year July 1, 1964 to June 30, 1965

Work & Line Project Number	Work and Line Project Titles	Work Location During Past Year	Line Proj. Incl. in Summary of Progress (Yes-No)	Area and Subheading
W5 5-37 (Rev.)	Evaluation of hydroxy-conjugated dienoic acid oils	Albany, Calif.	Yes	14-A-1
W5 5-46 (C)	Preparation and evaluation of surface coatings	Fargo, North Dakota	Yes	14-B-1
W6 1-41 (Rev.)[1]	Improvement of egg white products	Albany, Calif.	Yes	12-B-1
W6 1-48 (Rev.)	Chemistry of poultry flavor	Albany, Calif.	Yes	11-A-2
W6 1-49 (Rev.)[1]	Microbiology of cold-tolerant organisms	Albany, Calif.	Yes	12-B-1
W6 1-54[1]	Precooked frozen foods	Albany, Calif.	Yes	12-B-3
W6 1-55[1]	Improvement of yolk-containing egg solids	Albany, Calif.	Yes	12-B-2
W6 1-56[1]	Tenderness and other textural qualities of poultry meat	Albany, Calif.	Yes	11-A-1 11-B-2
W6 1-58 (C)[1]	Control of the neuromuscular retention and release of feathers	East Lansing, Michigan	Yes	11-A-3
W6 1-59 (C)	Reduction of Salmonella contamination in egg products	Ames, Iowa	Yes	12-B-1
W6 1-60 (C)	Histological study of frozen poultry	Madison, Wisc.	Yes	11-A-1
W6 1-61	Elimination of Salmonella in egg products	Albany, Calif.	Yes	12-B-1
W6 1-62	Freeze-dried poultry meat	Albany, Calif.	Yes	11-B-1
W6 1-64 (Gr.)	Salmonella metabolism	Ithaca, New York	Yes	12-B-1
W6 1-65 (C)	Freeze drying of poultry meat	Berkeley, Calif.	Yes	11-B-1
W6 1-66 (C)	Pasteurization of eggs	Davis, Calif.	Yes	12-B-1
W6 1-69 (C)	Chemical, physical or enzymic modification of egg white	Ames, Iowa	No[2]	
WU-P-1	Plant enzymes	Albany, Calif.	Yes	6-A-5
WU-0-0-1(BF)[1]	Hop oil flavor components	Albany, Calif.	Yes	8-A-1
WU-0-0-2(OCD)	Fallout shelter foods	Albany, Calif.	Yes	1-A-6 1-B-4
UR-A10-(10)-22	Rheology of wheat flour doughs	Haifa, Israel	Yes	1-A-2
UR-E9-(10)-2	Composition of whole wheat lipids	Paris, France	Yes	1-A-5
UR-E9-(10)-7	Immunochemical analysis of wheat and barley proteins	Paris, France	Yes	1-A-3
UR-E9-(10)-8	Solubility of wheat gluten proteins	Montpellier, France	Yes	1-A-3
UR-E9-(10)-43	Phosphorus in wheat flour	Paris, France	Yes	1-A-5
UR-E9-(10)-44	Ultrasonic study of wheat gluten	Paris, France	Yes	1-A-3
UR-E9-(10)-45	Enzyme action in low-moisture grain	Paris, France	Yes	1-A-5
UR-E15-(10)-31	Wheat germ proteins	Bologna, Italy	Yes	1-A-3
UR-E21-(10)-1	Sulfhydryl groups in wheat	Poznan, Poland	Yes	1-A-1
UR-E21-(10)-18	Coenzyme role of riboflavin of wheat endosperm	Poznan, Poland	Yes	1-A-4
UR-E27-(10)-1	Pentosans of wheat	Zurich, Switzerland	Yes	1-A-3

[1] Project discontinued during the reporting period.

[2] Recently initiated project.

Line Project Check List -- Reporting Year July 1, 1964 to June 30, 1965

Work & Line Project Number	Work and Line Project Titles	Work Location During Past Year	Line Proj. Incl. in Summary of Progress (Yes-No)	Area and Subheading
UR-E29-(10)-14[1]	Wheat flour lipids	Chorleywood, England	Yes	1-B-4
UR-E29-(10)-38	Separation of the total protein of wheat flour	St. Albans, England	Yes	1-A-3
UR-E29-(10)-47	Biological value of processed wheat	Cambridge, England	Yes	1-B-6
UR-E29-(10)-76	Bread dough interactions	Chorleywood, England	Yes	1-B-4
UR-01-(10)-1	Dough rheology	North Ryde, Australia	Yes	1-A-2
UR-E15-(10)-17	Natural antioxidants in alfalfa	Milano, Italy	Yes	3-A-2
UR-E29-(10)-52	Structure of alfalfa polysaccharides	Edinburgh, Scotland	Yes	3-A-3
UR-A7-(20)-15	Molecular processes in wool	Allahabad, India	Yes	4-A-1
UR-A7-(20)-58	Adsorption of selected ions to wool	Ahmedabad, India	No[2]	
UR-E8-(20)-10	Finishing treatments for improved qualities in wool fabrics	Helsinki, Finland	Yes	4-B-4
UR-E9-(20)-1[1]	Sequence of amino acids in wool proteins as related to quality differences	Lille, France	Yes	4-A-1
UR-E10-(20)-8	X-ray diffraction patterns of wool	Aachen, West Germany	No[2]	
UR-E26-(20)-7	Sulfur in wool keratins	Stockholm, Sweden	Yes	4-A-1
UR-E29-(20)-11[1]	Penetration of charged molecules into keratins	Leeds, England	Yes	4-A-1
UR-E29-(20)-22[1]	Lubrication of wool knitting yarns	Nottingham, England	Yes	4-B-3
UR-E29-(20)-56 (Rev.)	Chemical structure of wool protein	Leeds, England	Yes	4-A-1
UR-A6-(30)-3	Polysaccharides in plant cell walls	Taipei, Taiwan	Yes	6-A-3
UR-A7-(30)-60	Fruit leucoanthocyanins	Delhi, India	Yes	6-A-1
UR-A10-(30)-32	Enzymatic browning in deciduous fruits	Jerusalem, Israel	Yes	6-A-2
UR-S5-(30)-2	Tropical fruit flavors	Bogota, Colombia	Yes	5-A-3
UR-A7-(30)-39	Bean proteins	Allahabad, India	Yes	8-A-4
UR-E8-(10,30)-15	Composition of vegetables and fodder	Helsinki, Finland	Yes	8-A-1
UR-E9-(30)-54	Enzymatic activities of bacterial spores	Paris, France	Yes	8-A-2
UR-E26-(30)-5	Autoxidation of fats in dehydrated vegetables	Gothenburg, Sweden	Yes	7-A-1
UR-E26-(30)-11	Role of metals in vegetable enzyme action	Gothenburg,	No[2]	
UR-E29-(30)-16	Enzymatic browning of potato	Cambridge, England	Yes	7-A-2
UR-E29-(30)-17	Sulfur dioxide in dehydrated vegetables	London, England	No[3]	

1 Project discontinued during the reporting period.

2 Recently initiated project.

3 Research reported F.Y. 1964; project concluded July 1964.

Line Project Check List -- Reporting Year July 1, 1964 to June 30, 1965

Work & Line Project Number	Work and Line Project Titles	Work Location During Past Year	Line Proj. Incl. in Summary of Progress (Yes-No)	Area and Subheading
UR-A7-(40)-21	Hydroxylated derivatives of linseed and safflower oils	Hyderabad, India	No[4]	
UR-A7-(40)-69	Polymerizable monomers from castor oil	Hyderabad, India	No[2]	
UR-A7-(50)-31	Reaction of sucrose with sulfonyl chloride and other chemicals	Calcutta, India	Yes	10-B-2
UR-A10-(50)-25	Enzymatic sucrose degradation in sugar beet tissues	Jerusalem, Israel	Yes	10-A-1
UR-A7-(60)-27	Physicochemical properties of hen egg yolk proteins caused by freezing	Bangalore, India	Yes	12-A-1
UR-E9-(60)-76	Chemistry of egg lysozyme	Paris, France	Yes	12-A-1
UR-01-(60)-4	Ovalbumin in eggs	Ryde, New South Wales, Australia	Yes	12-A-1

2 Recently initiated project.

4 Joint project with Northern Utilization Research and Development Division. Only preliminary research has been done on safflower oil components.